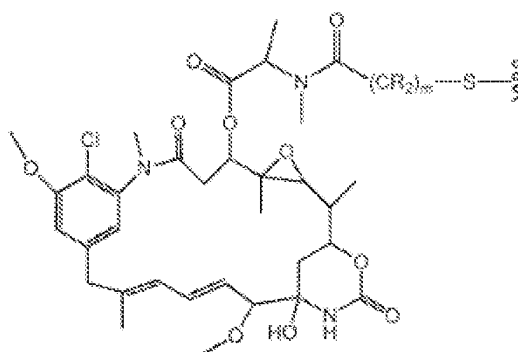


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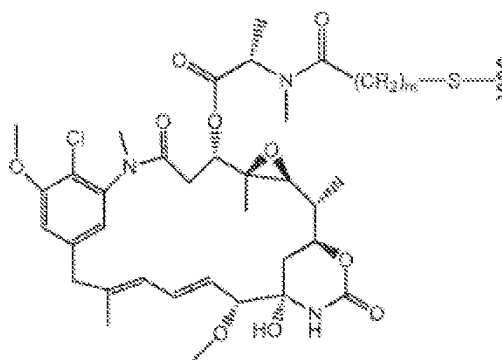
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where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid to a linker of an antibody drug conjugate. R at each occurrence is independently H or a C_1 - C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or proparyl, i.e. m is 1, 2, or 3. (U. S. Pat. No. 633,410, U.S. Pat. No. 5,208,020, Chari *et al.* (1992) Cancer Res. 52:127-131, Lui *et al.* (1996) Proc. Natl. Acad. Sci. 93:8618-8623).

[00152] All stereoisomers of the maytansinoid drug moiety are contemplated for the immunocjugates disclosed, *i.e.* any combination of R and S configurations at the chiral carbons of the maytansinoid. In one aspect the maytansinoid drug moiety has the following stereochemistry.

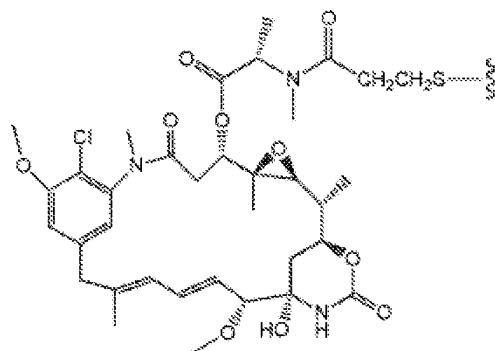


[00153] In one aspect, the maytansinoid drug moiety is N^Z -deacetyl- $N^{Z'}$ -(3-mercapto-1-oxopropyl)-maytansine (also known as DM1). DM1 is represented by the following structural formula.

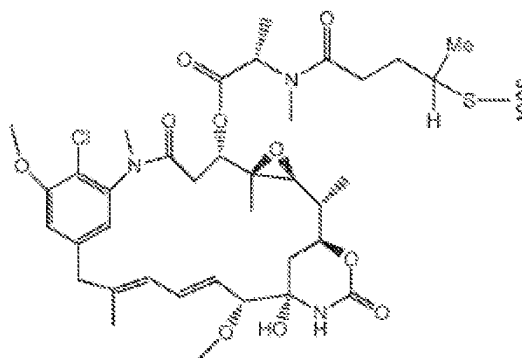
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**DM1**

[00154] In another aspect the maytansinoid drug moiety is N^7 -decetyl- N'' -(4-mercapto-1-oxopentyl)-maytansine (also known as DM3). DM3 is represented by the following structural formula.

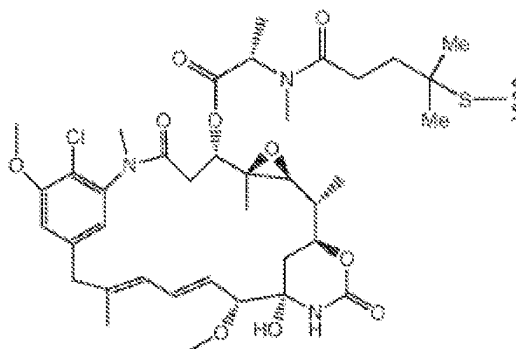
**DM3**

[00155] In another aspect the maytansinoid drug moiety is N^7 -decetyl- N'' -(4-methyl-4-mercapto-1-oxopentyl)-maytansine (also known as DM4). DM4 is represented by the following structural formula.

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**DM4**

[00156] The drug moiety D can be linked to the antibody Ab through a linker L. L is any chemical moiety that is capable of linking the antibody Ab to the drug moiety D. The linker, L, attaches the antibody Ab to the drug moiety D through covalent bond(s). The linker reagent is a bifunctional or multifunctional moiety which can be used to link a drug moiety D and an antibody Ab to form antibody drug conjugates. Antibody drug conjugates can be prepared using a linker having a reactive functionality for binding to the drug moiety D and to the antibody Ab. A cysteine, thiol or an amine, e.g. N-terminus or amino acid side chain such as lysine of the antibody can form a bond with a functional group of a linker reagent.

[00157] In one aspect, L is a cleavable linker. In another aspect, L is a non-cleavable linker. In some aspects, L is an acid-labile linker, photo-labile linker, peptidase cleavable linker, esterase cleavable linker, a disulfide bond reducible linker, a hydrophilic linker, a procharged linker, or a dicarboxylic acid based linker.

[00158] Suitable cross-linking reagents that form a non-cleavable linker between the drug moiety D, for example maytansinoid, and the antibody Ab are well known in the art, and can form non-cleavable linkers that comprise a sulfur atom (such as SMCC) or those that are without a sulfur atom. Preferred cross-linking reagents that form non-cleavable linkers between the drug moiety D, for example maytansinoid, and the antibody Ab comprise a maleimido- or haloacetyl-based moiety. According to the present disclosure, such non-cleavable linkers are said to be derived from maleimido- or haloacetyl-based moieties.

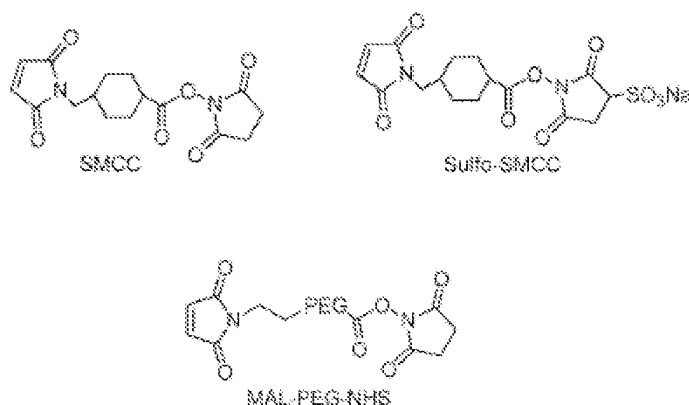
[00159] Cross-linking reagents comprising a maleimido-based moiety include but not limited to, *N*-succinimidyl-4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), *N*-succinimidyl-4-(maleimidomethyl)cyclohexane-1-carboxy-(6-aminocaproate), which is a "long chain" analog of

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SMCC (LC-SMCC), κ -maleimido- α -decanic acid *N*-succinimidyl ester (KMUA), γ -maleimido- α -butyric acid *N*-succinimidyl ester (GMBS), ϵ -maleimido- α -caproic acid *N*-succinimidyl ester (EMCS), *m*-maleimido-benzoyl-*N*-hydroxy-succinimide ester (MBS), *N*-(α -maleimido-acetoxy)-succinimide ester (AMSA), succinimidyl-6-(β -maleimido-propionamido)hexanoate (SMPH), *N*-succinimidyl-4-(*p*-maleimido-phenyl)-butyrate (SMPB), *N*-(*p*-maleimido-phenyl)isocyanate (PMIP) and maleimido-based cross-linking reagents containing a polyethylene glycol spacer, such as MAL-PEG-NHS. These cross-linking reagents form non-cleavable linkers derived from maleimido-based moieties. Representative structures of maleimido-based cross-linking reagents are shown below.



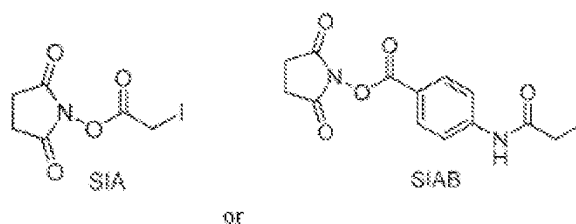
[00160] In another aspect, the linker L is derived from *N*-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) or MAL-PEG-NHS.

[00161] Cross-linking reagents comprising a haloacetyl-based moiety include *N*-succinimidyl iodoacetate (SIA), *N*-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), *N*-succinimidyl bromoacetate (SBA) and *N*-succinimidyl 3-(bromoacetamido)propionate (SBAP). These cross-linking reagents form a non-cleavable linker derived from haloacetyl-based moieties. Representative structures of haloacetyl-based cross-linking reagents are shown below.

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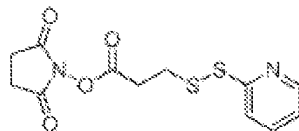
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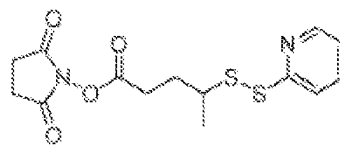


[00162] In one aspect, the linker L is derived from N-succinimidyl iodoacetate (SIA) or N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB).

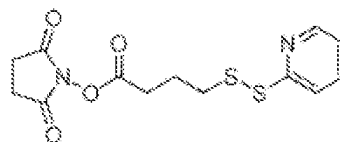
[00163] Suitable cross-linking reagents that form a cleavable linker between the drug moiety D, for example maytansinoid, and the antibody Ab are well known in the art. Disulfide containing linkers are linkers cleavable through disulfide exchange, which can occur under physiological conditions. According to the present disclosure, such cleavable linkers are said to be derived from disulfide-based moieties. Suitable disulfide cross-linking reagents include N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl-4-(2-pyridyldithio)butanoate (SPDB) and N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanone (sulfo-SPDB), the structures of which are shown below. These disulfide cross-linking reagents form a cleavable linker derived from disulfide-based moieties.



N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP),



N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP),

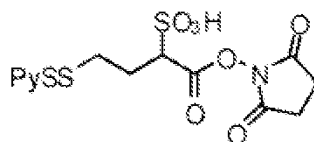


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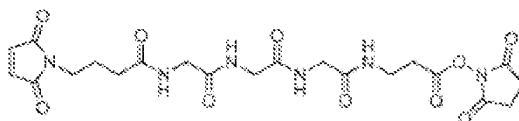
N-succinimidyl-4-(2-pyridyldithio)butanoate (SPDB) and



N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanoate (sulfo-SPDB)

[00164] In one aspect, the linker L is derived from N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanoate (sulfo-SPDB).

[00165] Suitable cross-linking reagents that form a charged linker between the drug moiety D, for example maytansinoid, and the antibody Ab are known as procharged cross-linking reagents. In one aspect, the linker L is derived from the procharged cross-linking reagent is CX1-1. The structure of CX1-1 is below.



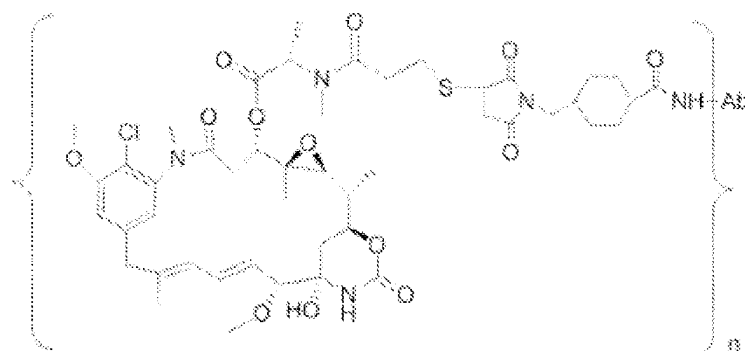
2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetrazaheptadecan-1-oate (CX1-1)

[00166] In one aspect provided by the disclosure, the conjugate is represented by any one of the following structural formulae:

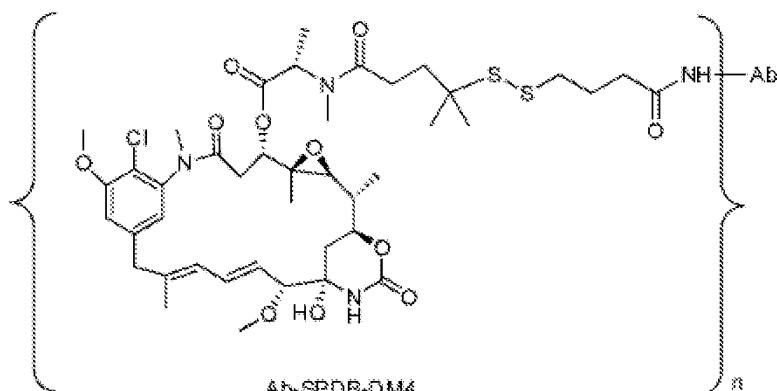
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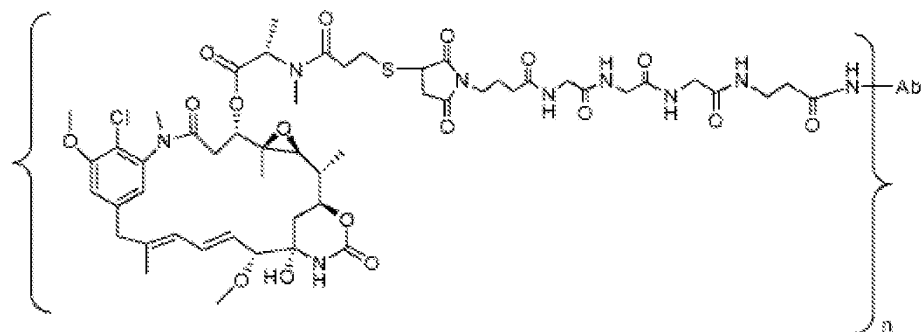
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Ab-MCC-DM1



Ab-SPDB-DM4

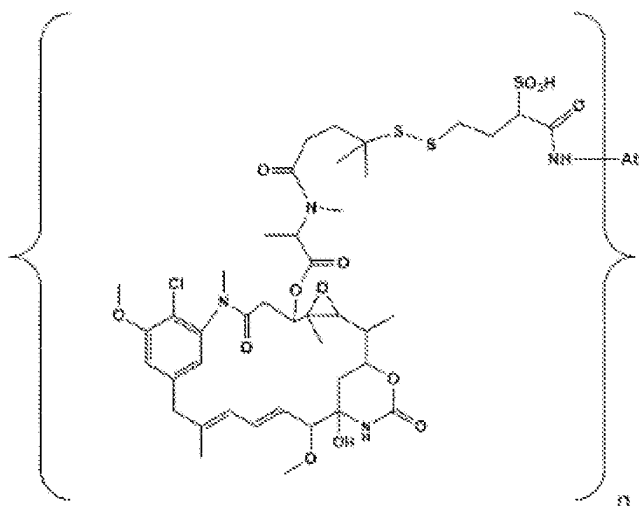


Ab-CX1-1-DM1

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Ab-sulfo-SPDB-DM4

[00167] wherein:

[00168] Ab is an antibody or antigen binding fragment thereof that specifically binds to human CDH6;

[00169] n, which indicates the number of D-L groups attached the Ab through the formation of an amide bond with a primary amine of the Ab, is an integer from 1 to 20. In one aspect, n is an integer from 1 to 10, 2 to 8 or 2 to 5. In a specific aspect, n is 3 or 4.

[00170] In one aspect, the average molar ratio of drug moiety (e.g., DM1 or DM4) to the antibody in the conjugate (i.e., average w value, also known as Maytansinoid Antibody Ratio (MAR)) is about 1 to about 10, about 2 to about 8 (e.g., 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, or 8.1), about 2.5 to about 7, about 3 to about 5, about 2.5 to about 4.5 (e.g., about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3.0, about 3.1, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5), about 3.0 to about 4.0, about 3.2 to about 4.2, or about 4.5 to 5.5 (e.g., about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, or about 5.5).

[00171] In one aspect provided by the disclosure, the conjugate has substantially high purity and has one or more of the following features: (a) greater than about 90% (e.g., greater than or equal to

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about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%), preferably greater than about 95%, of conjugate species are monomeric, (b) unconjugated linker level in the conjugate preparation is less than about 10% (*e.g.*, less than or equal to about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0%) (relative to total linker), (c) less than 10% of conjugate species are crosslinked (*e.g.*, less than or equal to about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0%), (d) free drug moiety (*e.g.*, DM1 or DM4) level in the conjugate preparation is less than about 2% (*e.g.*, less than or equal to about 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0%) (mol/mol relative to total cytotoxic agent).

[00172] As used herein, the term "unconjugated linker" refers to the antibody that is covalently linked with a linker derived from a cross-linking reagent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), wherein the antibody is not covalently coupled to the drug moiety (*e.g.*, DM1 or DM4) through a linker (*i.e.*, the "unconjugated linker" can be represented by Ab-SMCC, Ab-SPDB, Ab-sulfo-SPDB, or Ab-CX1-1).

1. Drug Moiety

[00173] The present disclosure provides immunoconjugates that specifically bind to CDH6. The immunoconjugates of the present disclosure comprise anti-CDH6 antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents that are conjugated to a drug moiety, *e.g.*, an anti-cancer agent, anti-hematological disorder agent, an autoimmune treatment agent, an anti-inflammatory agent, an antifungal agent, an antibacterial agent, an anti-parasitic agent, an anti-viral agent, or an anesthetic agent. The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents can be conjugated to several identical or different drug moieties using any methods known in the art.

[00174] In certain aspects, the drug moiety of the immunoconjugates of the present disclosure is selected from a group consisting of: a maytansinoid, a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an LAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, an inhibitor of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

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[00175] In one aspect, the drug moiety of the immunoconjugates of the present disclosure is a maytansinoid drug moiety, such as but not limited to, DM1, DM3, or DM4.

[00176] Further, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure may be conjugated to a drug moiety that modifies a given biological response. Drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein, peptide, or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, cholera toxin, or diphtheria toxin, a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a cytokine, an apoptotic agent, an anti-angiogenic agent, or, a biological response modifier such as, for example, a lymphokine.

[00177] In one aspect, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure are conjugated to a drug moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radiotoxin. Examples of cytotoxin include but are not limited to, taxanes (see, *e.g.*, International (PCT) Patent Application Nos. WO 01/38318 and PCT/US03/02675), DNA-alkylating agents (*e.g.*, CC-1065 analogs), anthracyclines, tubulysin analogs, duocarmycin analogs, auristatin E, auristatin F, maytansinoids, and cytotoxic agents comprising a reactive polyethylene glycol moiety (see, *e.g.*, Sasse *et al.*, *J. Antibiot. (Tokyo)*, 53, 879-85 (2000), Suzawa *et al.*, *Bioorg. Med. Chem.*, 8, 2175-84 (2000), Ichimuta *et al.*, *J. Antibiot. (Tokyo)*, 44, 1045-53 (1991), Francisco *et al.*, *Blood* 2003 15,102(4):1458-65), U.S. Pat. Nos. 5,475,092, 6,340,701, 6,372,738, and 6,436,931, U.S. Patent Application Publication No. 2001/0036923 A1, Pending U.S. patent application Ser. Nos. 10/024,290 and 10/116,053, and International (PCT) Patent Application No. WO 01/49698), taxon, cytochalasin B, gramicidin D, ethidium bromide, enetidine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1 -dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, anti-metabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), ablating agents (*e.g.*, mechlorethamine, thiotepe chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin, anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin

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(AMC), and anti-mitotic agents (*e.g.*, vincristine and vinblastine). (See *e.g.*, Seattle Genetics US20090304721).

[00178] Other examples of cytotoxins that can be conjugated to the antibodies, antibody fragments (antigen binding fragments) or functional equivalents of the present disclosure include duocarmycins, calicheamicins, maytansins and auristatins, and derivatives thereof.

[00179] Various types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies are known in the art, see, *e.g.*, Saito *et al.*, (2003) *Adv. Drug Deliv. Rev.* 55:199-215; Trail *et al.*, (2003) *Cancer Immunol. Immunother.* 52:328-337; Payne, (2003) *Cancer Cell* 3:207-212; Allen, (2002) *Nat. Rev. Cancer* 2:750-763; Pastan and Kreitman, (2002) *Curr. Opin. Investig. Drugs* 3:1089-1091; Senter and Springer, (2001) *Adv. Drug Deliv. Rev.* 53:247-264.

[00180] The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure can also be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine-131, indium-111, yttrium-90, and lutetium-177. Methods for preparing radioimmunoconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including ZevalinTM (IDEC Pharmaceuticals) and BexxarTM (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies disclosed herein. In certain aspects, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al.*, (1998) *Clin Cancer Res.* 4(10):2483-90; Peterson *et al.*, (1999) *Bioconjug. Chem.* 10(4):553-7; and Zimmerman *et al.*, (1999) *Nucl. Med. Biol.* 26(8):943-50, each incorporated by reference in their entireties.

[00181] The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure can also be conjugated to a heterologous protein or polypeptide (or fragment thereof, preferably to a polypeptide of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids) to generate fusion proteins. In particular, the present disclosure provides fusion proteins comprising an antibody fragment (*e.g.*, antigen binding fragment) described herein (*e.g.*, a Fab fragment, Fd fragment, Fv fragment, F(ab)₂ fragment, a VH domain, a VH CDR, a VL domain or a VL CDR) and a heterologous protein, polypeptide, or peptide.

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[00182] Additional fusion proteins may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to alter the activities of antibodies of the present disclosure or fragments thereof (e.g., antibodies or fragments thereof with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458; Patten *et al.*, (1997) *Curr. Opinion Biotechnol.* 8:724-33; Hatayama, (1998) *Trends Biotechnol.* 16(2):76-82; Hansson *et al.*, (1999) *J. Mol. Biol.* 287:265-76; and Lorenzo and Blasco, (1998) *Biotechniques* 24(2):308-313 (each of these patents and publications are hereby incorporated by reference in its entirety). Antibodies or fragments thereof, or the encoded antibodies or fragments thereof, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. A polynucleotide encoding an antibody or fragment thereof that specifically binds to an antigen may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[00183] Moreover, the antibodies, antibody fragments (e.g., antigen binding fragments) or functional equivalents of the present disclosure can be conjugated to marker sequences, such as a peptide, to facilitate purification. In preferred aspects, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Chatsworth, CA), among others, many of which are commercially available. As described in Gentz *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin ("HA") tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, (1984) *Cell* 37:767), and the "FLAG" tag (A. Einheber *et al.*, *J. Biochem. Biophys. Methods* 49: 455-465, 2001). As described in the present disclosure, antibodies or antigen binding fragments can also be conjugated to tumor-penetrating peptides in order to enhance their efficacy.

[00184] In other aspects, the antibodies, antibody fragments (e.g., antigen binding fragments) or functional equivalents of the present disclosure are conjugated to a diagnostic or detectable agent. Such immunoconjugates can be useful for monitoring or prognosing the onset, development, progression and/or severity of a disease or disorder as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can be accomplished by coupling the antibody to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin and

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avidin/biotin; fluorescent materials, such as, but not limited to, Alexa Fluor 350, Alexa Fluor 405, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 500, Alexa Fluor 514, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 610, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, Alexa Fluor 750, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as, but not limited to, iodine (^{125}I , ^{127}I , ^{131}I , and ^{132}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In , ^{113}In , ^{115}In , and ^{117}In), technetium (^{99}Tc), thallium (^{201}Tl), gallium (^{67}Ga , ^{68}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{166}Gd , ^{187}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{89}Y , ^{87}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{84}Sr , ^{32}P , ^{153}Gd , ^{90}Yb , ^{51}Cr , ^{54}Mn , ^{52}Fe , ^{64}Cu , ^{113}Sn , and ^{117}Sn ; and positron emitting metals using various positron emission tomographies, and non-radioactive paramagnetic metal ions.

[00185] The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present disclosure may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

2. Linker

[00186] As used herein, a "linker" is any chemical moiety that is capable of linking an antibody, antibody fragment (*e.g.*, antigen binding fragments) or functional equivalent to another moiety, such as a drug moiety. Linkers can be susceptible to cleavage (cleavable linker), such as, acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the compound or the antibody remains active. Alternatively, linkers can be substantially resistant to cleavage (*e.g.*, stable linker or noncleavable linker). In some aspects, the linker is a procharged linker, a hydrophilic linker, or a dicarboxylic acid based linker.

[00187] In one aspect, the linker used is derived from a crosslinking reagent such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl)

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cyclohexanecarboxylate (SMCC), *N*-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC) or 2,5-dioxypyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetrazapeptadecan-1-oate (CX1-1). In another aspect, the linker used is derived from a cross-linking agent such as *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), *N*-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), *N*-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC), *N*-succinimidyl 4-(2-pyridyldithio)-2-sulfo-butanate (sulfo-SPDB) or 2,5-dioxypyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetrazapeptadecan-1-oate (CX1-1).

[00188] Non-cleavable linkers are any chemical moiety capable of linking a drug moiety, such as a maytansinoid, to an antibody in a stable, covalent manner and does not fall off under the categories listed above for cleavable linkers. Thus, non-cleavable linkers are substantially resistant to acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage and disulfide bond cleavage. Furthermore, non-cleavable refers to the ability of the chemical bond in the linker or adjoining to the linker to withstand cleavage induced by an acid, photolabile-cleaving agent, a peptidase, an esterase, or a chemical or physiological compound that cleaves a disulfide bond, at conditions under which the drug moiety, such as maytansinoid or the antibody does not lose its activity.

[00189] Acid-labile linkers are linkers cleavable at acidic pH. For example, certain intracellular compartments, such as endosomes and lysosomes, have an acidic pH (pH 4-5), and provide conditions suitable to cleave acid-labile linkers.

[00190] Photo-labile linkers are linkers that are useful at the body surface and in many body cavities that are accessible to light. Furthermore, infrared light can penetrate tissue.

[00191] Some linkers can be cleaved by peptidases, i.e. peptidase cleavable linkers. Only certain peptides are readily cleaved inside or outside cells, see e.g. Trout et al., 79 Proc. Natl. Acad. Sci. USA, 626-629 (1982) and Umemoto et al. 43 Int. J. Cancer, 677-684 (1989). Furthermore, peptides are composed of α -amino acids and peptidic bonds, which chemically are amide bonds between the carboxylate of one amino acid and the amino group of a second amino acid. Other amide bonds, such as the bond between a carboxylate and the ϵ -amino group of lysine, are understood not to be peptidic bonds and are considered non-cleavable.

[00192] Some linkers can be cleaved by esterases, i.e. esterase cleavable linkers. Again, only certain esters can be cleaved by esterases present inside or outside of cells. Esters are formed by the

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condensation of a carboxylic acid and an alcohol. Simple esters are esters produced with simple alcohols, such as aliphatic alcohols, and small cyclic and small aromatic alcohols.

[00193] Procharged linkers are derived from charged cross-linking reagents that retain their charge after incorporation into an antibody drug conjugate. Examples of procharged linkers can be found in US 2009/0274713.

3. Conjugation and Preparation of ADCs

[00194] The conjugates of the present disclosure can be prepared by any methods known in the art, such as those described in US Patent Nos. 7,811,572, 6,411,163, 7,368,565, and 8,163,888, and US application publications 2011/0003969, 2011/0166319, 2012/0253021 and 2012/0259100. The entire teachings of these patents and patent application publications are herein incorporated by reference.

One-Step Process

[00195] In one aspect, the conjugates of the present disclosure can be prepared by a one-step process. The process comprises combining the antibody, drug and cross-linking agent in a substantially aqueous medium, optionally containing one or more co-solvents, at a suitable pH. In one aspect, the process comprises the step of contacting the antibody of the present disclosure with a drug (e.g., DM1 or DM4) to form a first mixture comprising the antibody and the drug, and then contacting the first mixture comprising the antibody and the drug with a cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate (e.g., Ab-MCC-DM1, Ab-SPDB-DM4, Sulfo-SPDB-DM4, or Ab-CX1-1-DM1), (ii) free drug (e.g., DM1 or DM4), and (iii) reaction by-products.

[00196] In one aspect, the one-step process comprises contacting the antibody with the drug (e.g., DM1 or DM4) and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 6 or greater (e.g., about 6 to about 9, about 6 to about 7, about 7 to about 9, about 7 to about 8.5, about 7.5 to about 8.5, about 7.5 to about 8.0, about 8.0 to about 9.0, or about 8.5 to about 9.0). For example, the process comprises contacting a cell-binding agent with the drug (DM1 or DM4) and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, or about 9.0. In another aspect, the process comprises contacting a cell-binding agent with the drug (e.g., DM1 or DM4) and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 7.8 (e.g., a pH of 7.6 to 8.0 or a pH of 7.7 to 7.9).

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[00197] The one-step process (i.e., contacting the antibody with the drug (e.g., DM1 or DM4) and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) can be carried out at any suitable temperature known in the art. For example, the one-step process can occur at about 20°C or less (e.g., about -10°C (provided that the solution is prevented from freezing, e.g., by the presence of organic solvent used to dissolve the cytotoxic agent and the bifunctional crosslinking reagent) to about 20°C, about 0°C to about 18°C, about 4°C to about 16°C), at room temperature (e.g., about 20°C to about 30°C or about 20°C to about 25°C), or at an elevated temperature (e.g., about 30°C to about 37°C). In one aspect, the one-step process occurs at a temperature of about 16°C to about 24°C (e.g., about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, or about 25°C). In another aspect, the one-step process is carried out at a temperature of about 15°C or less (e.g., about -10°C to about 15°C, or about 0°C to about 15°C). For example, the process comprises contacting the antibody with the drug (e.g., DM1 or DM4) and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at a temperature of about 15°C, about 14°C, about 13°C, about 12°C, about 11°C, about 10°C, about 9°C, about 8°C, about 7°C, about 6°C, about 5°C, about 4°C, about 3°C, about 2°C, about 1°C, about 0°C, about -1°C, about -2°C, about -3°C, about -4°C, about -5°C, about -6°C, about -7°C, about -8°C, about -9°C, or about -10°C, provided that the solution is prevented from freezing, e.g., by the presence of organic solvent(s) used to dissolve the cross-linking agent (e.g., SMCC, Sulfo-SMCC, Sulfo-SPDB, SPDB, or CX1-1). In one aspect, the process comprises contacting the antibody with the drug (e.g., DM1 or DM4) and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at a temperature of about -10°C to about 15°C, about 0°C to about 15°C, about 0°C to about 10°C, about 0°C to about 5°C, about 5°C to about 15°C, about 10°C to about 15°C, or about 5°C to about 10°C. In another aspect, the process comprises contacting the antibody with the drug (e.g., DM1 or DM4) and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at a temperature of about 10°C (e.g., a temperature of 8°C to 12°C or a temperature of 9°C to 11°C).

[00198] In one aspect, the contacting described above is effected by providing the antibody, then contacting the antibody with the drug (e.g., DM1 or DM4) to form a first mixture comprising the antibody and the drug (e.g., DM1 or DM4), and then contacting the first mixture comprising the antibody and the drug (e.g., DM1 or DM4) with the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). For example, in one aspect, the antibody is provided in a reaction vessel, the drug (e.g., DM1 or DM4) is added to the reaction vessel (thereby contacting the antibody), and then the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) is added to the mixture comprising the antibody and the drug (e.g., DM1 or DM4) (thereby contacting the mixture comprising the antibody and the drug). In one aspect, the antibody is provided in a reaction vessel, and the drug (e.g., DM1 or DM4) is added to the reaction vessel immediately following

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providing the antibody to the vessel. In another aspect, the antibody is provided in a reaction vessel, and the drug (*e.g.*, DM1 or DM4) is added to the reaction vessel after a time interval following providing the antibody to the vessel (*e.g.*, about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 1 day or longer after providing the cell-binding agent to the space). The drug (*e.g.*, DM1 or DM4) can be added quickly (*i.e.*, within a short time interval, such as about 5 minutes, about 10 minutes) or slowly (such as by using a pump).

[00199] The mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) can then be contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) either immediately after contacting the antibody with the drug (*e.g.*, DM1 or DM4) or at some later point (*e.g.*, about 5 minutes to about 8 hours or longer) after contacting the antibody with the drug (*e.g.*, DM1 or DM4). For example, in one aspect, the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) is added to the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) immediately after the addition of the drug (*e.g.*, DM1 or DM4) to the reaction vessel comprising the antibody. Alternatively, the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) can be contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, or longer after contacting the antibody with the drug (*e.g.*, DM1 or DM4).

[00200] After the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) the reaction is allowed to proceed for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, or longer (*e.g.*, about 30 hours, about 35 hours, about 40 hours, about 45 hours, or about 48 hrs).

[00201] In one aspect, the one-step process further comprises a quenching step to quench any unreacted drug (*e.g.*, DM1 or DM4) and/or unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). The quenching step is typically performed prior to purification of the conjugate. In one aspect, the mixture is quenched by contacting the mixture with a quenching reagent. As used herein, the "quenching reagent" refers to a reagent that reacts with the free drug (*e.g.*, DM1 or DM4) and/or cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). In one aspect, maleimide or haloacetamide quenching reagents, such as 4-maleimidobutyric acid, 3-maleimidopropionic acid, N-ethylmaleimide, iodoacetamide, or iodoacetamidopropionic acid, can be used to ensure that any unreacted group (such as thiol) in the drug (*e.g.*, DM1 or DM4) is quenched. The quenching step can help prevent the dimerization of the drug (*e.g.*, DM1). The dimerized DM1

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can be difficult to remove. Upon quenching with polar, charged thiol-quenching reagents (such as 4-maleimidobutyric acid or 3-maleimidopropionic acid), the excess, unreacted DMI is converted into a polar, charged, water-soluble adduct that can be easily separated from the covalently-linked conjugate during the purification step. Quenching with non-polar and neutral thiol-quenching reagents can also be used. In one aspect, the mixture is quenched by contacting the mixture with a quenching reagent that reacts with the unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). For example, nucleophiles can be added to the mixture in order to quench any unreacted SMCC. The nucleophile preferably is an amino group containing nucleophile, such as lysine, murine and hydroxylamine.

[00202] In another aspect, the reaction (*i.e.*, contacting the antibody with the drug (*e.g.*, DMI or DM4) and then cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1)) is allowed to proceed to completion prior to contacting the mixture with a quenching reagent. In this regard, the quenching reagent is added to the mixture about 1 hour to about 48 hours (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, or about 25 hours to about 48 hours) after the mixture comprising the antibody and the drug (*e.g.*, DMI or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1).

[00203] Alternatively, the mixture is quenched by lowering the pH of the mixture to about 5.0 (*e.g.*, 4.8, 4.9, 5.0, 5.1 or 5.2). In another aspect, the mixture is quenched by lowering the pH to less than 6.0, less than 5.5, less than 5.0, less than 4.8, less than 4.6, less than 4.4, less than 4.2, less than 4.0. Alternatively, the pH is lowered to about 4.0 (*e.g.*, 3.8, 3.9, 4.0, 4.1 or 4.2) to about 6.0 (*e.g.*, 5.8, 5.9, 6.0, 6.1 or 6.2), about 4.0 to about 5.0, about 4.5 (*e.g.*, 4.3, 4.4, 4.5, 4.6 or 4.7) to about 5.0. In one aspect, the mixture is quenched by lowering the pH of the mixture to 4.8. In another aspect, the mixture is quenched by lowering the pH of the mixture to 5.5.

[00204] In one aspect, the one-step process further comprises a holding step to release the unstably bound linkers from the antibody. The holding step comprises holding the mixture prior to purification of the conjugate (*e.g.*, after the reaction step, between the reaction step and the quenching step, or after the quenching step). For example, the process comprises (a) contacting the antibody with the drug (*e.g.*, DMI or DM4) to form a mixture comprising the antibody and the drug (*e.g.*, DMI or DM4); and then contacting the mixture comprising the antibody and drug (*e.g.*, DMI or DM4) with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate (*e.g.*, Ab-MCC-DMI, Ab-SPDB-DM4, Sulfo-SPDB-DM4 or Ab-CX1-1-DMI), (ii) free drug (*e.g.*, DMI or DM4), and (iii) reaction by-products, (b) holding the mixture prepared in step (a) to release the unstably

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bound linkers from the cell-binding agent, and (c) purifying the mixture to provide a purified conjugate.

[00205] In another aspect, the process comprises (a) contacting the antibody with the drug (*e.g.*, DM1 or DM4) to form a mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4), and then contacting the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate, (ii) free drug (*e.g.*, DM1 or DM4), and (iii) reaction by-products, (b) quenching the mixture prepared in step (a) to quench any unreacted drug (*e.g.*, DM1 or DM4) and/or unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), (c) holding the mixture prepared in step (b) to release the unstably bound linkers from the cell-binding agent, and (d) purifying the mixture to provide a purified conjugate (*e.g.*, Ab-MCC-DM1, Ab-SPDB-DM4, Ab-Sulfo-SPDB-DM4 or Ab-CX1-1-DM1).

[00206] Alternatively, the holding step can be performed after purification of the conjugate, followed by an additional purification step.

[00207] In another aspect, the reaction is allowed to proceed to completion prior to the holding step. In this regard, the holding step can be performed about 1 hour to about 48 hours (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, or about 24 hours to about 48 hours) after the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1).

[00208] The holding step comprises maintaining the solution at a suitable temperature (*e.g.*, about 0°C to about 37°C) for a suitable period of time (*e.g.*, about 1 hour to about 1 week, about 1 hour to about 24 hours, about 1 hour to about 8 hours, or about 1 hour to about 4 hours) to release the unstably bound linkers from the antibody while not substantially releasing the stably bound linkers from the antibody. In one aspect, the holding step comprises maintaining the solution at about 20 °C or less (*e.g.*, about 0°C to about 18°C, about 4°C to about 16°C), at room temperature (*e.g.*, about 20°C to about 30°C or about 20°C to about 25°C), or at an elevated temperature (*e.g.*, about 30°C to about 37°C). In one aspect, the holding step comprises maintaining the solution at a temperature of about 16°C to about 24°C (*e.g.*, about 15°C, about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, or about 25°C). In another aspect, the holding step comprises maintaining the solution at a temperature of about 2°C to about 8°C (*e.g.*, about 0°C, about 1°C, about 2°C, about 3°C, about 4°C, about 5°C, about 6°C, about 7°C, about 8°C, about 9°C, or about 10°C). In another aspect, the holding step comprises maintaining the solution at a temperature of

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about 37°C (*e.g.*, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, or about 40°C).

[00209] The duration of the holding step depends on the temperature and the pH at which the holding step is performed. For example, the duration of the holding step can be substantially reduced by performing the holding step at elevated temperature, with the maximum temperature limited by the stability of the cell-binding agent-cytotoxic agent conjugate. The holding step can comprise maintaining the solution for about 1 hour to about 1 day (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, or about 24 hours), about 10 hours to about 24 hours, about 12 hours to about 24 hours, about 14 hours to about 24 hours, about 16 hours to about 24 hours, about 18 hours to about 24 hours, about 20 hours to about 24 hours, about 5 hours to about 1 week, about 20 hours to about 1 week, about 12 hours to about 1 week (*e.g.*, about 12 hours, about 16 hours, about 20 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days), or about 1 day to about 1 week.

[00210] In one aspect, the holding step comprises maintaining the solution at a temperature of about 2 °C to about 8 °C for a period of at least about 12 hours for up to a week. In another aspect, the holding step comprises maintaining the solution at a temperature of about 2 °C to about 8 °C overnight (*e.g.*, about 12 to about 24 hours, preferably about 20 hours).

[00211] The pH value for the holding step preferably is about 4 to about 10. In one aspect, the pH value for the holding step is about 4 or more, but less than about 6 (*e.g.*, 4 to 5.9) or about 5 or more, but less than about 6 (*e.g.*, 5 to 5.9). In another aspect, the pH values for the holding step range from about 6 to about 10 (*e.g.*, about 6.5 to about 9, about 6 to about 8). For example, pH values for the holding step can be about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10.

[00212] In other aspects, the holding step can comprise incubating the mixture at 25°C at a pH of about 6-7.5 for about 12 hours to about 1 week, incubating the mixture at 4°C at a pH of about 4.5-5.9 for about 5 hours to about 5 days, or incubating the mixture at 25°C at a pH of about 4.5-5.9 for about 5 hours to about 1 day.

[00213] The one-step process can optionally include the addition of sucrose to the reaction step to increase solubility and recovery of the conjugates. Desirably, sucrose is added at a concentration of about 0.1% (w/v) to about 20% (w/v) (*e.g.*, about 0.1% (w/v), 1% (w/v), 5% (w/v), 10% (w/v), 15% (w/v), or 20% (w/v)). Preferably, sucrose is added at a concentration of about 1% (w/v) to about 10% (w/v) (*e.g.*, about 0.5% (w/v), about 1% (w/v), about 1.5% (w/v), about 2% (w/v), about 3% (w/v), about 4% (w/v), about 5% (w/v), about 6% (w/v), about 7% (w/v), about 8% (w/v), about 9% (w/v), about 10% (w/v), or about 11% (w/v)). In addition, the reaction step also can

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comprise the addition of a buffering agent. Any suitable buffering agent known in the art can be used. Suitable buffering agents include, for example, a citrate buffer, an acetate buffer, a succinate buffer, and a phosphate buffer. In one aspect, the buffering agent is selected from the group consisting of HEPPSO (N-(2-hydroxyethyl)piperazine-N'-(2-hydroxypropanesulfonic acid)), POPSO (piperazine-1,4-bis-(2-hydroxy-propane-sulfonic acid) dehydrate), HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), HEPPS (EPPS) (4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid), TES (N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid), and a combination thereof.

[00214] The one-step process can further comprise the step of purifying the mixture to provide purified conjugate (e.g., Ab-MCC-DM1, Ab-SPDB-DM4, Ab-Sulfo-SPDB-DM4 or Ab-CX1-1-DM1). Any purification methods known in the art can be used to purify the conjugates of the present disclosure. In one aspect, the conjugates of the present disclosure use tangential flow filtration (TFF), non-adsorptive chromatography, adsorptive chromatography, adsorptive filtration, selective precipitation, or any other suitable purification process, as well as combinations thereof. In another aspect, prior to subjecting the conjugates to purification process described above, the conjugates are first filtered through one or more PVDF membranes. Alternatively, the conjugates are filtered through one or more PVDF membranes after subjecting the conjugates to the purification process described above. For example, in one aspect, the conjugates are filtered through one or more PVDF membranes and then purified using tangential flow filtration. Alternatively, the conjugates are purified using tangential flow filtration and then filtered through one or more PVDF membranes.

[00215] Any suitable TFF systems may be utilized for purification, including a Pellicon® type system (Millipore, Billerica, MA), a Sartocoon® Cassette system (Sartorius AG, Edgewood, NY), and a Centrasette® type system (Pall Corp., East Hills, NY).

[00216] Any suitable adsorptive chromatography resin may be utilized for purification. Preferred adsorptive chromatography resins include hydroxyapatite chromatography, hydrophobic charge induction chromatography (HCIC), hydrophobic interaction chromatography (HIC), ion exchange chromatography, mixed mode ion exchange chromatography, immobilized metal affinity chromatography (IMAC), dye ligand chromatography, affinity chromatography, reversed phase chromatography, and combinations thereof. Examples of suitable hydroxyapatite resins include ceramic hydroxyapatite (CHF Type I and Type II, Bio-Rad Laboratories, Hercules, CA), HA Ultrogel® hydroxyapatite (Pall Corp., East Hills, NY), and ceramic fluoroapatite (CFT Type I and Type II, Bio-Rad Laboratories, Hercules, CA). An example of a suitable HCIC resin is MEP Hypercel® resin (Pall Corp., East Hills, NY). Examples of suitable HIC resins include Butyl-Sepharose, Hexyl-Sepharose, Phenyl-Sepharose, and Octyl-Sepharose resins (all from GE Healthcare, Piscataway, NJ), as well as Macro-prep® Methyl and Macro-Prep® t-Butyl resins (Biorad Laboratories, Hercules, CA). Examples of suitable ion exchange resins include SP-Sepharose®, CM-Sepharose®, and Q-Sepharose® resins (all from GE Healthcare, Piscataway, NJ), and Unosphere® S

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resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable mixed mode ion exchangers include Bakerbond® ABx resin (JT Baker, Phillipsburg NJ). Examples of suitable IMAC resins include Chelating Sepharose® resin (GE Healthcare, Piscataway, NJ) and Profinity® IMAC resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable dye ligand resins include Blue Sepharose resin (GE Healthcare, Piscataway, NJ) and Affi-gel Blue resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable affinity resins include Protein A Sepharose resin (*e.g.*, MabSelect, GE Healthcare, Piscataway, NJ) and lectin affinity resins, *e.g.*, Lentil Lectin Sepharose® resin (GE Healthcare, Piscataway, NJ), where the antibody bears appropriate lectin binding sites. Examples of suitable reversed phase resins include C4, C8, and C18 resins (Grace Vydac, Hesperia, CA).

[00217] Any suitable non-adsorptive chromatography resin may be utilized for purification. Examples of suitable non-adsorptive chromatography resins include, but are not limited to, SEPHADEX™ G-25, G-50, G-100, SEPHACRYL™ resins (*e.g.*, S-200 and S-300), SUPERDEX™ resins (*e.g.*, SUPERDEX™ 75 and SUPERDEX™ 200), BIO-GEL® resins (*e.g.*, P-6, P-10, P-30, P-60, and P-100), and others known to those of ordinary skill in the art.

Two-Step Process and One-Pot Process

[00218] In one aspect, the conjugates of the present disclosure can be prepared as described in the U.S. Patent 7,811,572 and U.S. Patent Application Publication No. 2006/0182750. The process comprises the steps of (a) contacting the antibody of the present disclosure with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) to covalently attach the linker (*i.e.*, Ab-SMCC, Ab-SPDB or Ab-CX1-1) to the antibody and thereby prepare a first mixture comprising the antibody having the linker bound thereto; (b) optionally subjecting the first mixture to a purification process to prepare a purified first mixture of the antibody having the linker bound thereto; (c) conjugating the drug (*e.g.*, DM1 or DM4) to the antibody having the linker bound thereto in the first mixture by reacting the antibody having the linker bound thereto with the drug (*e.g.*, DM1 or DM4) in a solution having a pH of about 4 to about 9 to prepare a second mixture comprising (i) conjugate (*e.g.*, Ab-MCC-DM1, Ab-SPDB-DM4, Ab-Sulfo-SPDB-DM4 or Ab-CX1-1-DM1), (ii) free drug (*e.g.*, DM1 or DM4); and (iii) reaction by-products; and (d) subjecting the second mixture to a purification process to purify the conjugate from the other components of the second mixture. Alternatively, the purification step (b) can be omitted. Any purification methods described herein can be used for steps (b) and (d). In one embodiment, TFF is used for both steps (b) and (d). In another embodiment, TFF is used for step (b) and absorptive chromatography (*e.g.*, CHT) is used for step (d).

One-Step Reagent and In-situ Process

[00219] In one aspect, the conjugates of the present disclosure can be prepared by conjugating pre-formed drug-linker compound (*e.g.*, SMCC-DM1, Sulfo-SMCC-DM1, SPDB-DM4, Sulfo-SPDB-

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DM4 or CX1-1-DM1) to the antibody of the present disclosure, as described in U.S. Patent 6,431,163 and U.S. Patent Application Publication Nos. 2011/0003969 and 2008/0145374, followed by a purification step. Any purification methods described herein can be used. The drug-linker compound is prepared by reacting the drug (e.g., DM1 or DM4) with the cross-linking agent (e.g., SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). The drug-linker compound (e.g., SMCC-DM1, Sulfo-SMCC-DM1, SPDB-DM4, Sulfo-SPDB-DM4 or CX1-1-DM1) is optionally subjected to purification before being conjugated to the antibody.

4. Characterization and Selection of Desirable Antibodies and Antibody Drug Conjugates

[00220] The antibodies, antibody fragments (e.g., antigen binding fragments) or antibody drug conjugates of the present disclosure can be characterized and selected for their physical/chemical properties and/or biological activities by various assays known in the art.

[00221] For example, an antibody of the present disclosure can be tested for its antigen binding activity by known methods such as ELISA, FACS, Biacore or Western blot.

[00222] Transgenic animals and cell lines are particularly useful in screening antibody drug conjugates (ADCs) that have potential as prophylactic or therapeutic treatments of cancer overexpression of tumor-associated antigens and cell surface receptors. Screening for a useful ADC may involve administering a candidate ADC over a range of doses to the transgenic animal, and assaying at various time points for the effect(s) of the ADC on the disease or disorder being evaluated. Alternatively, or additionally, the drug can be administered prior to or simultaneously with exposure to an inducer of the disease, if applicable. The candidate ADC may be screened serially and individually, or in parallel under medium or high-throughput screening format.

[00223] One aspect is a screening method comprising (a) transplanting cells from a stable cancer cell line or human patient tumor expressing CDH6 (e.g., an ovarian cell line or tumor fragment, a renal cell line or tumor fragment, a hepatic cell line or tumor fragment, a thyroid cell line or tumor fragment, a CNS cancer cell line or tumor fragment, a cholangiocarcinoma cancer cell line or tumor fragment, ovarian, renal, hepatic, soft tissue, CNS, thyroid, or cholangiocarcinoma primary cells) into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the growth of tumors from the transplanted cell line. The present disclosure also encompasses a method of screening ADC candidates for the treatment of a disease or disorder characterized by the overexpression of CDH6 comprising (a) contacting cells from a stable cancer cell line expressing CDH6 with a drug candidate, and (b) evaluating the ability of the ADC candidate to inhibit the growth of the stable cell line.

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[00224] A further aspect is a screening method comprising (a) contacting cells from a stable cancer cell line expressing CDH6 with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one aspect the ability of the ADC candidate to induce apoptosis is evaluated.

[00225] Candidate ADC can be screened by being administered to the transgenic animal over a range of doses, and evaluating the animal's physiological response to the compounds over time. In some cases, it can be appropriate to administer the compound in conjunction with co-factors that would enhance the efficacy of the compound. If cell lines derived from the subject transgenic animals are used to screen for ADCs useful in treating various disorders associated with overexpression of CDH6, the test ADCs are added to the cell culture medium at an appropriate time, and the cellular response to the ADCs is evaluated over time using the appropriate biochemical and/or histological assays.

[00226] Thus, the present disclosure provides assays for identifying ADC which specifically target and bind to CDH6, and CDH6 expressed on tumor cells.

CDH6 Antibodies

[00227] The present disclosure provides for antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to human CDH6. Antibodies or antibody fragments (e.g., antigen binding fragments) of the present disclosure include, but are not limited to, the human monoclonal antibodies or fragments thereof, isolated as described, in the Examples below.

[00228] The present disclosure in certain aspects provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind CDH6, said antibodies or antibody fragments (e.g., antigen binding fragments) comprise a VH domain having an amino acid sequence of SEQ ID NO: 20, 34, 48, 62, 76, 90, 104, 118, 132, 146, 160, 174, 188, 202, 216, 230, 244, 258, 272, 286, 300, 314 or 328 (Table 5 and 6). The present disclosure also provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments (e.g., antigen binding fragments) comprise a VH CDR having an amino acid sequence of any one of the VH CDRs listed in Table 5 and 6. In particular aspects, the present disclosure provides antibodies or antibody fragments (e.g., antigen binding fragments) that specifically bind to CDH6, said antibodies comprising (or alternatively, consist of) one, two, three, four, five or more VH CDRs having an amino acid sequence of any of the VH CDRs listed in Table 5 and 6.

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[00229] The present disclosure provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise a VL domain having an amino acid sequence of SEQ ID NO: 21, 35, 49, 63, 77, 91, 105, 119, 133, 147, 161, 175, 189, 203, 217, 231, 245, 259, 273, 287, 301, 315 or 329 (Table 5 and 6). The present disclosure also provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise a VL CDR having an amino acid sequence of any one of the VL CDRs listed in Table 5 and 6, *infra*. In particular, the disclosure provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to CDH6, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise (or alternatively, consist of) one, two, three or more VL CDRs having an amino acid sequence of any of the VL CDRs listed in Table 5 and 6.

[00230] Other antibodies or antibody fragments (*e.g.*, antigen binding fragments) of the present disclosure include amino acids that have been mutated, yet have at least 60, 70, 80, 90 or 95 percent identity in the CDR regions with the CDR regions depicted in the sequences described in Table 5 and 6. In some aspects, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the CDR regions when compared with the CDR regions depicted in the sequence described in Table 5 and 6.

[00231] The present disclosure also provides nucleic acid sequences that encode VH, VL, the full length heavy chain, and the full length light chain of the antibodies that specifically bind to CDH6. Such nucleic acid sequences can be optimized for expression in mammalian cells.

[00232] Other antibodies of the present disclosure include those where the amino acids or nucleic acids encoding the amino acids have been mutated, yet have at least 60, 70, 80, 90 or 95 percent identity to the sequences described in 5 and 6. In some aspects, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the variable regions when compared with the variable regions depicted in the sequence described in 5 and 6, while retaining substantially the same therapeutic activity.

[00233] Since each of these antibodies can bind to CDH6, the VH, VL, full length light chain, and full length heavy chain sequences (amino acid sequences and the nucleotide sequences encoding the amino acid sequences) can be "mixed and matched" to create other CDH6-binding antibodies. Such "mixed and matched" CDH6-binding antibodies can be tested using the binding assays known in the art (*e.g.*, ELISAs, and other assays described in the Example section). When these chains are mixed and matched, a VH sequence from a particular VH/VL pairing should be replaced with a

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structurally similar VH sequence. Likewise a full length heavy chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length heavy chain sequence. Likewise, a VL sequence from a particular VH/VL pairing should be replaced with a structurally similar VL sequence. Likewise, a full length light chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length light chain sequence. Accordingly, in one aspect, the disclosure provides for an isolated monoclonal antibody or antigen binding region thereof having: a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 34, 48, 62, 76, 90, 104, 118, 132, 146, 160, 174, 188, 202, 216, 230, 244, 258, 272, 286, 300, 314 or 328 (Table 5 and 6); and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, 35, 49, 63, 77, 91, 105, 119, 133, 147, 161, 175, 189, 203, 217, 231, 245, 259, 273, 287, 301, 315 or 329 (Table 5 and 6); wherein the antibody specifically binds to CDH6.

[00234] In another aspect, the disclosure provides (i) an isolated monoclonal antibody having: a full length heavy chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NO: 24, 38, 52, 66, 80, 94, 108, 122, 136, 150, 164, 178, 192, 206, 220, 234, 248, 262, 276, 290, 304, 318 or 332; and a full length light chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NO: 25, 39, 53, 67, 81, 95, 109, 123, 137, 151, 165, 179, 193, 207, 221, 235, 249, 263, 277, 291, 305, 319 or 333; or (ii) a functional protein comprising an antigen binding portion thereof.

[00235] In another aspect, the present disclosure provides CDH6-binding antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s as described in Table 5 and 6, or combinations thereof. The amino acid sequences of the VH CDR1s of the antibodies are shown in SEQ ID NOs: 17, 31, 45, 59, 73, 87, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 241, 255, 269, 283, 297, 311 and 325. The amino acid sequences of the VH CDR2s of the antibodies are shown in SEQ ID NOs: 18, 32, 46, 60, 74, 88, 102, 116, 130, 144, 158, 172, 186, 200, 214, 228, 242, 256, 270, 284, 298, 312 and 326. The amino acid sequences of the VH CDR3s of the antibodies are shown in SEQ ID NOs: 19, 33, 47, 61, 75, 89, 103, 117, 131, 145, 159, 173, 187, 201, 215, 229, 243, 257, 271, 285, 299, 313 and 327. The amino acid sequences of the VL CDR1s of the antibodies are shown in SEQ ID NOs: 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182, 196, 210, 224, 238, 252, 266, 280, 294, 308 and 322. The amino acid sequences of the VL CDR2s of the antibodies are shown in SEQ ID NOs: 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, 267,

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281, 295, 309 and 323. The amino acid sequences of the VL CDR3s of the antibodies are shown in SEQ ID NOs: 16, 30, 44, 58, 72, 86, 100, 114, 128, 142, 156, 170, 184, 198, 212, 226, 240, 254, 268, 282, 296, 310 and 324.

[00236] Given that each of these antibodies can bind to CDH6 and that antigen-binding specificity is provided primarily by the CDR1, 2 and 3 regions, the VH CDR1, 2 and 3 sequences and VL CDR1, 2 and 3 sequences can be "mixed and matched" (i.e., CDRs from different antibodies can be mixed and match, although each antibody must contain a VH CDR1, 2 and 3 and a VL CDR1, 2 and 3 to create other CDH6-binding molecules. Such "mixed and matched" CDH6-binding antibodies can be tested using the binding assays known in the art and those described in the Examples (*e.g.*, ELISAs). When VH CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VH sequence should be replaced with a structurally similar CDR sequence(s). Likewise, when VL CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VL sequence should be replaced with a structurally similar CDR sequence(s). It will be readily apparent to the ordinarily skilled artisan that novel VH and VL sequences can be created by substituting one or more VH and/or VL CDR region sequences with structurally similar sequences from the CDR sequences shown herein for monoclonal antibodies of the present disclosure.

[00237] Accordingly, the present disclosure provides an isolated monoclonal antibody or antigen binding region thereof comprising a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 31, 45, 59, 73, 87, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 241, 255, 269, 283, 297, 311 and 325; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 32, 46, 60, 74, 88, 102, 116, 130, 144, 158, 172, 186, 200, 214, 228, 242, 256, 270, 284, 298, 312 and 326; a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 33, 47, 61, 75, 89, 103, 117, 131, 145, 159, 173, 187, 201, 215, 229, 243, 257, 271, 285, 299, 313 and 327; a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, 154, 168, 182, 196, 210, 224, 238, 252, 266, 280, 294, 308 and 322; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, 267, 281, 295, 309 and 323; and a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 30, 44, 58, 72, 86, 100, 114, 128, 142, 156, 170, 184, 198, 212, 226, 240, 254, 268, 282, 296, 310 and 324; wherein the antibody specifically binds CDH6.

[00238] In a specific aspect, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to CDH6 comprising:

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- (i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;
- (ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;
- (iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;
- (iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;
- (v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:14, (b) a LCDR2 of SEQ ID NO:15, (c) a LCDR3 of SEQ ID NO:16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:17, (e) a HCDR2 of SEQ ID NO:18, and (f) a HCDR3 of SEQ ID NO:19;
- (vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;
- (vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;
- (viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;
- (ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

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- (x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;
- (xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO:100; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:101, (e) a HCDR2 of SEQ ID NO:102, and (f) a HCDR3 of SEQ ID NO:103;
- (xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:112, (b) a LCDR2 of SEQ ID NO:113, (c) a LCDR3 of SEQ ID NO:114; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:115, (e) a HCDR2 of SEQ ID NO:116, and (f) a HCDR3 of SEQ ID NO:117;
- (xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:126, (b) a LCDR2 of SEQ ID NO:127, (c) a LCDR3 of SEQ ID NO:128; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:129, (e) a HCDR2 of SEQ ID NO:130, and (f) a HCDR3 of SEQ ID NO:131;
- (xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:140, (b) a LCDR2 of SEQ ID NO:141, (c) a LCDR3 of SEQ ID NO:142; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:143, (e) a HCDR2 of SEQ ID NO:144, and (f) a HCDR3 of SEQ ID NO:145;
- (xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:154, (b) a LCDR2 of SEQ ID NO:155, (c) a LCDR3 of SEQ ID NO:156; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:157, (e) a HCDR2 of SEQ ID NO:158, and (f) a HCDR3 of SEQ ID NO:159;
- (xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:168, (b) a LCDR2 of SEQ ID NO:169, (c) a LCDR3 of SEQ ID NO:170; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:171, (e) a HCDR2 of SEQ ID NO:172, and (f) a HCDR3 of SEQ ID NO:173;
- (xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:182, (b) a LCDR2 of SEQ ID NO:183, (c) a LCDR3 of SEQ ID NO:184; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:185, (e) a HCDR2 of SEQ ID NO:186, and (f) a HCDR3 of SEQ ID NO:187;
- (xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:196, (b) a LCDR2 of SEQ ID NO:197, (c) a LCDR3 of SEQ ID NO:198; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

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(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

[00239] In certain aspects, an antibody that specifically binds to CDH6 is an antibody or antibody fragment (*e.g.*, antigen binding fragment) that is described in Table 5 and/or 6.

1. Identification of Epitopes and Antibodies That Bind to the Same Epitope

[00240] The present disclosure provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that bind to an epitope of within the extracellular domain of CDH6. In certain aspects the antibodies and antibody fragments can bind to epitopes within domains of the CDH6 extracellular domain.

[00241] The present disclosure also provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that bind to the same epitope as do the anti-CDH6 antibodies described in Table 5 and 6. Additional antibodies and antibody fragments (*e.g.*, antigen binding fragments) can therefore be identified based on their ability to cross-compete (*e.g.*, to competitively inhibit the binding of, in a statistically significant manner) with other antibodies in CDH6 binding assays. The ability of a test antibody to inhibit the binding of antibodies and antibody fragments (*e.g.*, antigen binding fragments)

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of the present disclosure to a CDH6 protein (*e.g.*, human CDH6) demonstrates that the test antibody can compete with that antibody or antibody fragment (*e.g.*, antigen binding fragments) for binding to CDH6; such an antibody may, according to non-limiting theory, bind to the same or a related (*e.g.*, a structurally similar or spatially proximal) epitope on the CDH6 protein as the antibody or antibody fragment (*e.g.*, antigen binding fragments) with which it competes. In a certain aspect, the antibody that binds to the same epitope on CDH6 as the antibodies or antibody fragments (*e.g.*, antigen binding fragments) of the present disclosure is a human or humanized monoclonal antibody. Such human or humanized monoclonal antibodies can be prepared and isolated as described herein.

2. Further Alteration of the Framework of Fc Region

[00242] The present disclosure provides site-specific labeled immunoconjugates. These immunoconjugates can comprise modified antibodies or antigen binding fragments thereof that further comprise modifications to framework residues within VH and/or VL, *e.g.* to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "back-mutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. To return the framework region sequences to their germline configuration, the somatic mutations can be "back-mutated" to the germline sequence by, for example, site-directed mutagenesis. Such "back-mutated" antibodies are also intended to be encompassed.

[00243] Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 2003/0153043 by Carr *et al.*

[00244] In addition or alternative to modifications made within the framework or CDR regions, antibodies can be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody can be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or be modified to alter

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its glycosylation, again to alter one or more functional properties of the antibody. Each of these aspects is described in further detail below.

[00245] In one aspect, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

[00246] In another aspect, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcal protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

[00247] In yet other aspects, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in, *e.g.*, U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

[00248] In another aspect, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in, *e.g.*, U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

[00249] In another aspect, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described in, *e.g.*, the PCT Publication WO 94/29351 by Bodmer *et al.* In a specific aspect, one or more amino acids of an antibody or antigen binding fragment thereof of the present disclosure are replaced by one or more allotypic amino acid residues, for the IgG1 subclass and the kappa isotype. Allotypic amino acid residues also include, but are not limited to, the constant region of the heavy chain of the IgG1, IgG2, and IgG3 subclasses as well as the constant region of the light chain of the kappa isotype as described by Jefferis *et al.*, MAbs. 1:332-338 (2009).

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[00250] In yet another aspect, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids. This approach is described in, *e.g.*, the PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn have been mapped and variants with improved binding have been described (see Shields *et al.*, J. Biol. Chem. 276:6591-6604, 2001).

[00251] In still another aspect, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for "antigen." Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in, *e.g.*, U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

[00252] Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields *et al.*, (2002) J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umara *et al.* describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNAc structures which results in increased ADCC activity of the antibodies (see also Umara *et al.*, Nat. Biotech. 17:176-180, 1999).

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[00253] In another aspect, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

[00254] In order to minimize the ADCC activity of an antibody, specific mutations in the Fc region result in "Fc silent" antibodies that have minimal interaction with effector cells. In general, the "IgG Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain, including native sequence Fc region and variant Fc regions. The human IgG heavy chain Fc region is generally defined as comprising the amino acid residue from position C226 or from P230 to the carboxyl-terminus of the IgG antibody. The numbering of residues in the Fc region is that of the EU index of Kabat. The C-terminal lysine (residue K447) of the Fc region may be removed, for example, during production or purification of the antibody.

[00255] Silenced effector functions can be obtained by mutation in the Fc region of the antibodies and have been described in the art: LALA and N297A (Strohl, W., 2009, Curr. Opin. Biotechnol. vol. 20(6):685-691); and D265A (Bardino et al., 2008, J. Immunol. 181 : 6664-69) see also Heusser et al., WO2012065950. Examples of silent Fc IgG1 antibodies are the LALA mutant comprising L234A and L235A mutation in the IgG1 Fc amino acid sequence. Another example of a silent IgG1 antibody is the DAPA (D265A, P329A) mutation (US 6,737,056). Another silent IgG1 antibody comprises the N297A mutation, which results in aglycosylated/non-glycosylated antibodies.

[00256] Fc silent antibodies result in no or low ADCC activity, meaning that an Fc silent antibody exhibits an ADCC activity that is below 50% specific cell lysis. No ADCC activity means that the Fc silent antibody exhibits an ADCC activity (specific cell lysis) that is below 1 %.

3. Production of the CDH6 Antibodies

[00257] Anti-CDH6 antibodies and antibody fragments (*e.g.*, antigen binding fragments) thereof can be produced by any means known in the art, including but not limited to, recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full-length monoclonal antibodies can be obtained by, *e.g.*, hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc.

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[00258] The disclosure further provides polynucleotides encoding the antibodies described herein, *e.g.*, polynucleotides encoding heavy or light chain variable regions or segments comprising the complementarity determining regions as described herein. In some aspects, the polynucleotide encoding the heavy chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 22, 36, 50, 64, 78, 92, 106, 120, 134, 148, 162, 176, 190, 204, 218, 232, 246, 260, 274, 288, 302, 316, and 330. In some aspects, the polynucleotide encoding the light chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 23, 37, 51, 65, 79, 93, 107, 121, 135, 149, 163, 177, 191, 205, 219, 233, 247, 261, 275, 289, 303, 317, and 331.

[00259] In some aspects, the polynucleotide encoding the heavy chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 26, 40, 54, 68, 82, 96, 110, 124, 138, 152, 166, 180, 194, 208, 222, 236, 250, 264, 278, 292, 306, 320, and 334. In some aspects, the polynucleotide encoding the light chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 27, 41, 55, 69, 83, 97, 111, 125, 139, 153, 167, 181, 195, 209, 223, 237, 251, 265, 279, 293, 307, 321, and 335.

[00260] The polynucleotides of the present disclosure can encode only the variable region sequence of an anti-CDH6 antibody. They can also encode both a variable region and a constant region of the antibody. Some of the polynucleotide sequences encode a polypeptide that comprises variable regions of both the heavy chain and the light chain of one of an exemplified anti-CDH6 antibody. Some other polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of the heavy chain and the light chain of one of the mouse antibodies.

[00261] The polynucleotide sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (*e.g.*, sequences as described in the Examples below) encoding an anti-CDH6 antibody or its binding fragment. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang *et al.*, *Meth. Enzymol.* 68:90, 1979; the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68:109, 1979; the diethylphosphoramidite method of Beaucage *et al.*, *Tetrahedron Lett.*, 22:1859, 1981; and the solid support method of U.S. Patent No. 4,458,066. Introducing mutations to a polynucleotide sequence by

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PCR can be performed as described in, *e.g.*, PCR Technology: Principles and Applications for DNA Amplification, H.A. Erlich (Ed.), Freeman Press, NY, NY, 1992; PCR Protocols: A Guide to Methods and Applications, Innis *et al.* (Ed.), Academic Press, San Diego, CA, 1990; Mattila *et al.*, Nucleic Acids Res. 19:967, 1991; and Eckert *et al.*, PCR Methods and Applications 1:17, 1991.

[00262] Also provided in the present disclosure are expression vectors and host cells for producing the anti-CDH6 antibodies described above. Various expression vectors can be employed to express the polynucleotides encoding the anti-CDH6 antibody chains or binding fragments. Both viral-based and nonviral expression vectors can be used to produce the antibodies in a mammalian host cell. Nonviral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial chromosomes (see, *e.g.*, Harrington *et al.*, Nat Genet. 15:345, 1997). For example, nonviral vectors useful for expression of the anti-CDH6 polynucleotides and polypeptides in mammalian (*e.g.*, human) cells include pThioHis A, B & C, pcDNA3.1/His, pEBVHis A, B & C (Invitrogen, San Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing other proteins. Useful viral vectors include vectors based on retroviruses, adenoviruses, adenoassociated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBV Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent *et al.*, *supra*; Smith, Annu. Rev. Microbiol. 49:807, 1995; and Rosenfeld *et al.*, Cell 68:143, 1992.

[00263] The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (*e.g.*, enhancers) that are operably linked to the polynucleotides encoding an anti-CDH6 antibody chain or fragment. In some aspects, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, *e.g.*, arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under noninducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of an anti-CDH6 antibody chain or fragment. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, *e.g.*, Scharf *et al.*, Results Probl. Cell Differ. 20:125, 1994; and Bitner *et al.*, Meth. Enzymol., 153:516, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

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[00264] The expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted anti-CDH6 antibody sequences. More often, the inserted anti-CDH6 antibody sequences are linked to a signal sequences before inclusion in the vector. Vectors to be used to receive sequences encoding anti-CDH6 antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies or fragments thereof. Typically, such constant regions are human.

[00265] The host cells for harboring and expressing the anti-CDH6 antibody chains can be either prokaryotic or eukaryotic. *E. coli* is one prokaryotic host useful for cloning and expressing the polynucleotides of the present disclosure. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (*trp*) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express anti-CDH6 polypeptides. Insect cells in combination with baculovirus vectors can also be used.

[00266] In other aspects, mammalian host cells are used to express and produce the anti-CDH6 polypeptides of the present disclosure. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes (*e.g.*, a myeloma hybridoma clone) or a mammalian cell line harboring an exogenous expression vector (*e.g.*, SP2/0 myeloma cells). These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed, including the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, *e.g.*, Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, *e.g.*, Queen *et al.*, *Immunol. Rev.* 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites,

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and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMfTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

[00267] Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts (see generally Sambrook *et al.*, *supra*). Other methods include, *e.g.*, electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation/nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, Cell 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express anti-CDH6 antibody chains or binding fragments can be prepared using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate to the cell type.

Therapeutic and Diagnostic Uses

[00268] The antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates of the present disclosure are useful in a variety of applications including, but not limited to, treatment of cancer, such as solid cancers. In certain aspects, the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates are useful for inhibiting tumor growth, inducing differentiation, reducing tumor volume, and/or reducing the tumorigenicity of a tumor. The methods of use can be *in vitro*, *ex vivo*, or *in vivo* methods.

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[00269] In one aspect, the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates are useful for detecting the presence of CDH6 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain aspects, a biological sample comprises a cell or tissue. In certain aspects, such tissues include normal and/or cancerous tissues that express CDH6 at higher levels relative to other tissues.

[00270] In one aspect, the present disclosure provides a method of detecting the presence of CDH6 in a biological sample. In certain aspects, the method comprises contacting the biological sample with an anti-CDH6 antibody under conditions permissive for binding of the antibody to the antigen, and detecting whether a complex is formed between the antibody and the antigen.

[00271] Also included is a method of diagnosing a disorder associated with increased expression of CDH6. In certain aspects, the method comprises contacting a test cell with an anti-CDH6 antibody; determining the level of expression (either quantitatively or qualitatively) of CDH6 on the test cell by detecting binding of the anti-CDH6 antibody to the CDH6 antigen; and comparing the level of expression of CDH6 in the test cell with the level of expression of CDH6 in a control cell (*e.g.*, a normal cell of the same tissue origin as the test cell or a cell that expresses CDH6 at levels comparable to such a normal cell), wherein a higher level of expression of CDH6 on the test cell as compared to the control cell indicates the presence of a disorder associated with increased expression of CDH6. In certain aspects, the test cell is obtained from an individual suspected of having a disorder associated with increased expression of CDH6. In certain aspects, the disorder is a cell proliferative disorder, such as a cancer or a tumor.

[00272] In certain aspects, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-CDH6 antibody to CDH6 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing CDH6 on its surface. An exemplary assay for detecting binding of an anti-CDH6 antibody to CDH6 expressed on the surface of a cell is a "FACS" assay.

[00273] Certain other methods can be used to detect binding of anti-CDH6 antibodies to CDH6. Such methods include, but are not limited to, antigen-binding assays that are well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

[00274] In certain aspects, anti-CDH6 antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-

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dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction.

[00275] In certain aspects, anti-CDH6 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-CDH6 antibody from any CDH6 proteins that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-CDH6 antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al, U.S. Patent No. 3,729,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the anti-CDH6 antibody after formation of a complex between the anti-CDH6 antibody and CDH6 protein, *e.g.*, by immunoprecipitation.

[00276] Any of the above aspects of diagnosis or detection can be carried out using an immunoconjugate of the present disclosure in place of or in addition to an anti-CDH6 antibody.

[00277] In one aspect, the disclosure provides for a method of treating, preventing or ameliorating a disease comprising administering the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates to a patient, thereby treating the disease. In certain aspects, the disease treated with the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates is a cancer. Examples of diseases which can be treated and/or prevented include, but are not limited to, ovarian cancer, renal cancer, hepatic cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma. In certain aspects, the cancer is characterized by CDH6 expressing cells to which the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates can specifically bind.

[00278] The present disclosure provides for methods of treating cancer comprising administering a therapeutically effective amount of the antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates. In certain aspects, the cancer is a solid cancer. In certain aspects, the subject is a human.

[00279] In certain aspects, the method of inhibiting tumor growth comprises administering to a subject a therapeutically effective amount of the antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates. In certain aspects, the subject is a human. In certain aspects, the subject has a tumor or has had a tumor removed.

[00280] In certain aspects, the tumor expresses the CDH6 to which the anti-CDH6 antibody binds. In certain aspects, the tumor overexpresses the human CDH6.

[00281] For the treatment of the disease, the appropriate dosage of the antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates depend on various factors,

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such as the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, previous therapy, patient's clinical history, and so on. The antibody or agent can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (e.g., reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual antibody, antibody fragment (e.g., antigen binding fragment), or antibody drug conjugates. In certain aspects, dosage is from 0.01 mg to 10 mg (e.g., 0.01 mg, 0.05 mg, 0.1 mg, 0.5 mg, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 7 mg, 8 mg, 9 mg, or 10 mg) per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. In certain aspects, the antibody, antibody fragment (e.g., antigen binding fragment), or antibody drug conjugate of the present disclosure is given once every two weeks or once every three weeks. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

Combination Therapy

[00282] In certain instances, an antibody, antibody fragment (e.g., antigen binding fragment), or antibody drug conjugate of the present disclosure is combined with other therapeutic agents, such as other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

[00283] General chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex[®]), bicalutamide (Casodex[®]), bleomycin sulfate (Blenoxane[®]), busulfan (Myleran[®]), busulfan injection (Busulfex[®]), capecitabine (Xeloda[®]), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin[®]), carmustine (BiCNU[®]), chlorambucil (Leukeran[®]), cisplatin (Platinol[®]), cladribine (Leustatin[®]), cyclophosphamide (Cytosan[®] or Neosar[®]), cytarabine, cytosine arabinoside (Cytosar-U[®]), cytarabine liposome injection (DepoCyt[®]), dacarbazine (DTIC-Dome[®]), dactinomycin (Actinomycin D, Cosmegen[®]), daunorubicin hydrochloride (Cerubidine[®]), daunorubicin citrate liposome injection (DaunoXome[®]), dexamethasone, docetaxel (Taxotere[®]), doxorubicin hydrochloride (Adriamycin[®], Rubex[®]), etoposide (Vepesid[®]), fludarabine phosphate (Fludara[®]), 5-fluorouracil (Adrucil[®], Efudex[®]), flutamide (Eulexin[®]), tezacitabine, Gemcitabine (difluorodeoxycytidine), hydrosyrea (Hydrea[®]), Idarubicin (Idamycin[®]), ifosfamide (IFEX[®]), irinotecan (Camptosar[®]), L-asparaginase (ELSPAR[®]), leucovorin calcium, melphalan (Alkeran[®]), 6-mercaptopurine (Purinethol[®]), methotrexate (Folex[®]), mitoxantrone (Novantrone[®]), mylotarg, paclitaxel (Taxol[®]), phoenix (Vitrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine

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implant (Gliadel[®]), tamoxifen citrate (Nolvadex[®]), teniposide (Vumon[®]), 6-thioguanine, thiotepe, tirapazamine (Tirazone[®]), topotecan hydrochloride for injection (Hycamptin[®]), vinblastine (Velban[®]), vincristine (Oncovin[®]), and vinorelbine (Navelbine[®]).

[00284] In one aspect, an antibody, antibody fragment (*e.g.*, antigen binding fragment), or antibody drug conjugate of the present disclosure is combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with a second compound having anti-cancer properties. The second compound of the pharmaceutical combination formulation or dosing regimen can have complementary activities to the antibody or immunoconjugate of the combination such that they do not adversely affect each other. For example, an antibody, antibody fragment (*e.g.*, antigen binding fragment), or antibody drug conjugate of the present disclosure can be administered in combination with, but not limited to, a chemotherapeutic agent, a tyrosine kinase inhibitor, for example, Imatinib.

[00285] The term "pharmaceutical combination" as used herein refers to either a fixed combination in one dosage unit form, or non-fixed combination or a kit of parts for the combined administration where two or more therapeutic agents may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, *e.g.* synergistic effect.

[00286] The term "combination therapy" refers to the administration of two or more therapeutic agents to treat a therapeutic condition or disorder described in the present disclosure. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of active ingredients. Alternatively, such administration encompasses co-administration in multiple, or in separate containers (*e.g.*, capsules, powders, and liquids) for each active ingredient. Powders and/or liquids may be reconstituted or diluted to a desired dose prior to administration. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner, either at approximately the same time or at different times. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the conditions or disorders described herein.

[00287] The combination therapy can provide "synergy" and prove "synergistic", *i.e.*, the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect can be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some

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other regimen. When delivered in alternation therapy, a synergistic effect can be attained when the compounds are administered or delivered sequentially, e.g., by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, *i.e.*, serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

[00288] In one aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more tyrosine kinase inhibitors, including but not limited to, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGF1R inhibitors, and Met inhibitors.

[00289] For example, tyrosine kinase inhibitors include but are not limited to, Erlotinib hydrochloride (Tarceva®); Linafinib (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea, also known as ABT 869, available from Genentech); Sunitinib malate (Sutent®); Bosutinib (4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile, also known as SKI-606, and described in US Patent No. 6,780,996); Dasatinib (Sprycel®); Pazopanib (Votrient®); Sorafenib (Nexavar®); Zactima (ZD6474); nilotinib (Tasigna®); Regorafenib (Stivarga®) and Imatinib or Imatinib mesylate (Gleevec® and Gleevec®).

[00290] Epidermal growth factor receptor (EGFR) inhibitors include but are not limited to, Erlotinib hydrochloride (Tarceva®), Gefitinib (Iressa®), N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[[O³S⁺]-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butanamide, Tovok®), Vandetanib (Caprelsa®), Lapatinib (Tykerb®); (3R,4R)-4-Amino-1-((4-(3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methylpiperidin-3-ol (BMS690314); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-1-piperazinyl)methyl]phenyl]-N-[(1R)-1-phenylethyl]-7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mabritinib (TAK165); Pelatinib (EKB569); Afatinib (BIBW2992); Neratinib (HKI-272); N-[4-[[1-(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamate acid, (3S)-3-morpholinylmethyl ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[[3 α ,5 β ,6 α]-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); and 4-[4-[[[(1R)-1-Phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol (PK1166, CAS 187724-61-4).

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[00291] EGFR antibodies include but are not limited to, Cetuximab (Erbixx®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Nimotuzumab (hR3); Zolatumumab; TheraCIM h-R3; MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1).

[00292] Human Epidermal Growth Factor Receptor 2 (HER2 receptor) (also known as Neu, ErbB-2, CD340, or p185) inhibitors include but are not limited to, Trastuzumab (Herceptin®); Pertuzumab (Omnitarg®); Neratinib (HKI-272, (2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide, and described PCT Publication No. WO 05/028443); Lapatinib or Lapatinib ditosylate (Tykerb®); (3R,4R)-4-amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidine-3-ol (BMS696514); (2E)-N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[3S]-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4-(dimethylamino)-2-butanamide (BIBW-2992, CAS 850140-72-6); N-[4-[[1-(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamate acid, (3S)-3-morpholinylmethyl ester (BMS 599626, CAS 714971-09-2); Canertinib dihydrochloride (PD183805 or CI-1033); and N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[3aa,5β,6aa]-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8).

[00293] HER3 inhibitors include but are not limited to, LJM716, MM-121, AMG-888, RG7116, REGN-1400, AV-203, MP-RM-1, MM-111, and MEHD-7945A.

[00294] MET inhibitors include but are not limited to, Cabozantinib (XL184, CAS 849217-68-1); Foretinib (GSK1363089, formerly XL880, CAS 849217-64-7); Tivantinib (ARQ197, CAS 1060873-98-2); 1-(2-Hydroxy-2-methylpropyl)-N-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxamide (AMG 458); Cryzotinib (Xalkori®), PF-02341066); (3Z)-5-(2,3-Dihydro-1H-indol-1-ylsulfonyl)-3-((3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl)methylene)-1,3-dihydro-2H-indol-2-one (SU11271); (3Z)-N-(3-Chlorophenyl)-3-((3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1H-pyrrol-2-yl)methylene)-N-methyl-2-oxindoline-5-sulfonamide (SU11274); (3Z)-N-(3-Chlorophenyl)-3-[[3,5-dimethyl-4-(3-morpholin-4-ylpropyl)-1H-pyrrol-2-yl]methylene]-N-methyl-2-oxindoline-5-sulfonamide (SU11606); 6-[Difluoro[6-(1-methyl-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-b]pyridazin-3-yl]methyl]-quinoline (JNJ38877605, CAS 943540-75-8); 2-[4-[1-(Quinolin-6-ylmethyl)-1H-[1,2,3]triazolo[4,5-b]pyrimidin-6-yl]-1H-pyrazol-1-yl]ethanol (PF04217903, CAS 956905-27-4); N-((2R)-1,4-Dioxan-2-ylmethyl)-N-methyl-N'-[3-(1-methyl-1H-pyrazol-4-yl)-5-oxo-5H-benzo[4,5]cyclohepta[1,2-b]pyridin-7-yl]sulfamide (MK2461, CAS 917879-39-1); 6-[[6-(1-Methyl-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-

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b[pyridazin-3-yl]thio]-quinoline (SGX523, CAS 1022150-57-7); and (3Z)-5-[(2,6-Dichlorophenyl)methyl]sulfonyl]-3-[[3,5-dimethyl-4-[(2*R*)-2-(1-pyrrolidinylmethyl)-1-pyrrolidinyl]carbonyl]-1*H*-pyrrol-2-yl]methylene]-1,3-dihydro-2*H*-indol-2-one (PHA665752, CAS 477575-56-7).

[00295] IGF1R inhibitors include but are not limited to, BMS-754807, XL-228, OSI-906, GSK0904529A, A-928605, AXL1717, KW-2450, MK0646, AMG479, IMCA12, MEDI-573, and BI836845. See e.g., Yee, JNCL 104; 975 (2012) for review.

[00296] In another aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more PGF downstream signaling pathway inhibitors, including but not limited to, MEK inhibitors, Raf inhibitors, PI3K/Akt inhibitors, SHP2 inhibitors, and also mTor inhibitors.

[00297] For example, mitogen-activated protein kinase (MEK) inhibitors include but are not limited to, XL-518 (also known as GDC-0973, Cas No. 1029872-29-4, available from ACC Corp.); 2-[(2-Chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluoro-benzamide (also known as CI-1040 or PD184352 and described in PCT Publication No. WO2000035436); N-[(2*R*)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide (also known as PD0325901 and described in PCT Publication No. WO2002006213); 2,3-Bis[amino(2-aminophenyl)thio]methylene]-butanedinitrile (also known as U0126 and described in US Patent No. 2,779,780); N-[3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]-6-methoxyphenyl]-1-[(2*R*)-2,3-dihydroxypropyl]-cyclopropanesulfonamide (also known as RDEA119 or BAY869766 and described in PCT Publication No. WO2007014011); (3*S*,4*R*,5*Z*,8*S*,9*S*,11*E*)-14-(Ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9,19-tetrahydro-1*H*-2-benzoxacyclotetradecine-1,7(8*H*)-dione] (also known as E6201 and described in PCT Publication No. WO2003076424); 2'-Amino-3'-methoxyflavone (also known as PD98059 available from Bioaffin GmbH & Co., KG, Germany); Vemurafenib (PLX-4032, CAS 918304-65-1); (R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-*d*]pyrimidine-4,7(3*H*,8*H*)-dione (TAK-733, CAS 1035555-63-5); Pimasertib (AS-703026, CAS 1204531-26-9); and Trametinib dimethyl sulfoxide (GSK-1120212, CAS 1204531-25-80).

[00298] Phosphoinositide 3-kinase (PI3K) inhibitors include but are not limited to, 4-[2-(1*H*-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-*d*]pyrimidin-4-yl]morpholine (also known as GDC 0941 and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-*c*]quinolin-1-

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yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasartib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolonyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione (PX866, CAS 592632-66-8); and 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6).

[00299] mTOR inhibitors include but are not limited to, Temsirolimus (Torisel®); Ridaferolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-2-[(1R,9S,12S,15R,16E,18R,19R,21R, 23S,24E,26E,28Z,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,26-pentaoxo-11,36-dioxo-4-azatricyclo[30.3.1.0^{4,9}] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or RAD001); Rapamycin (AY 22989, Sirolimus®); Simipimod (CAS 164301-51-3); (5-{2,4-Bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and *N*²-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L- α -aspartyl-L-serine-, inner salt (SF1126, CAS 936487-67-1).

[00300] In yet another aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more pro-apoptotics, including but not limited to, IAP inhibitors, Bcl2 inhibitors, Mcl1 inhibitors, Trail agents, Cdk inhibitors.

[00301] For examples, IAP inhibitors include but are not limited to, NVP-LCL161, GDC-0917, AEG-35156, AT406, and TL32711. Other examples of IAP inhibitors include but are not limited to those disclosed in WO04/005284, WO 04/007529, WO05/097791, WO 05/066984, WO 05/069888, WO 05/094818, US2006/0014700, US2006/0025347, WO 06/069063, WO 06/010118, WO 06/017295, and WO08/134679, all of which are incorporated herein by reference.

[00302] BCL-2 inhibitors include but are not limited to, 4-{4-[[2-(4-Chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl]methyl]-1-piperazinyl}-N-[[4-[[[(1R)-3-(4-morpholinyl)-1-

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[(phenylthio)methyl]propyl]amino]-3-[(trifluoromethyl)sulfonyl]phenyl[sulfonyl]benzamide (also known as ABT-263 and described in PCT Publication No. WO 09/155386); Tetrocarcin A; Antimycin; Gossypol ((-)-BL-193); Obatoclax; Ethyl-2-amino-6-cyclopentyl-4-(1-cyano-2-ethoxy-2-oxoethyl)-4H-chromone-3-carboxylate (HA14 – 1); Oblimersen (G3139, Genasense®); Bak BH3 peptide; (-)-Gossypol acetic acid (AT-101); 4-{4-[4'-Chloro[1,1'-biphenyl]-2-yl)methyl]-1-piperazinyl}-N-[[4-]](1R)-3-(dimethylamino)-1-[(phenylthio)methyl]propyl]amine]-3-nitrophenyl[sulfonyl]-benzamide (ABT-737, CAS 852808-04-9); and Navitoclax (ABT-263, CAS 923564-51-6).

[00303] Proapoptotic receptor agonists (PARAs) including DR4 (TRAILR1) and DR5 (TRAILR2), including but are not limited to, Dulanermin (AMG-951, RhApo2L/TRAIL); Mapatumumab (HRS-ETR1, CAS 658052-09-6); Lexatumumab (HGS-ETR2, CAS 845816-02-6); Apomab (Apomab®); Conatumumab (AMG655, CAS 896731-82-1); and Tigatuzumab (CS1008, CAS 946415-34-5, available from Daiichi Sankyo).

[00304] Checkpoint Kinase (CHK) inhibitors include but are not limited to, 7-Hydroxystaurosporine (UCN-01); 6-Bromo-3-(1-methyl-1H-pyrazol-4-yl)-5-(3H)-3-piperidinyl-pyrazolo[1,5-a]pyrimidin-7-amine (SCH900776, CAS 891494-63-6); 5-(3-Fluorophenyl)-3-ureidothiophene-2-carboxylic acid N-[(5S)-piperidin-3-yl]amide (AZD7762, CAS 860352-01-8); 4-[(3S)-1-Azabicyclo[2.2.2]oct-3-yl]amino]-3-(1H-benzimidazol-2-yl)-6-chloroquinolin-2(1H)-one (CHIR 124, CAS 405168-58-3); 7-Aminodactinomycin (7-AAD); Isogranulatimide; debromohymenialdisine; N-[5-Bromo-4-methyl-2-[(2S)-2-morpholinymethoxy]-phenyl]-N'-(5-methyl-2-pyrazinyl)urea (LY2603618, CAS 911222-45-2); Sulforaphane (CAS 4478-93-7, 4-Methylsulfinylbutyl isothiocyanate); 9,10,11,12-Tetrahydro-9,12-epoxy-1H-diindole[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6]benzodiazocine-1,3(2H)-dione (SB-218078, CAS 135897-06-2); and TAT-S216A (Sha et al., Mol. Cancer Ther 2007; 6(1):147-153), and CBP501.

[00305] In one aspect, the present disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with one or more EGFR inhibitors. For example, EGFR inhibitors include but are not limited to, Brivanib alaninate (BMS-582664, (S)-((R)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-amino)propanoate); Vargatef (BIBF1120, CAS 928326-83-4); Dovitinib dilactate acid (TKI258, CAS 852433-84-2); 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethylpiperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea (BGJ398, CAS 872511-34-7); Damsisertib (PHA-739358); and (PD173074, CAS 219580-13-7). In a specific aspect, the present

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disclosure provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate in combination with an EGFR2 inhibitor, such as 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (also known as BGJ-398); or 4-amino-5-fluoro-3-(5-(4-methylpiperazin-1-yl)-1*H*-benzo[d]imidazole-2-yl)quinolin-2(1*H*)-one (also known as denvitinib or TKI-258). AZD4547 (Gavine *et al.*, 2012, Cancer Research 72, 2045-56, N-[5-[2-(3,5-Dimethoxyphenyl)ethyl]-2*H*-pyrazol-3-yl]-4-(3*R*,5*S*)-diethyl(piperazin-1-yl)benzamide), Ponatinib (AP24534; Gozgit *et al.*, 2012, Mol Cancer Ther., 11; 690-99; 3-[2-(imidazo[1,2-*b*]pyridazin-3-yl)ethyl]nyl]-4-methyl-N-[4-[(4-methylpiperazin-1-yl)methyl]-5-(trifluoromethyl)phenyl]benzamide, CAS 943319-70-8)

Pharmaceutical Compositions

[00306] To prepare pharmaceutical or sterile compositions including immunoconjugates, the immunoconjugates of the present disclosure are mixed with a pharmaceutically acceptable carrier or excipient. The compositions can additionally contain one or more other therapeutic agents that are suitable for treating or preventing cancer, for example, ovarian cancer, renal cancer, hepatic cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma.

[00307] Formulations of therapeutic and diagnostic agents can be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, *e.g.*, Hardman *et al.*, Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y., 2001; Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y., 2000; Avis, *et al.* (eds.), Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY, 1993; Lieberman, *et al.* (eds.), Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY, 1990; Lieberman, *et al.* (eds.) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY, 1990; Weiner and Korkoskie, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y., 2000).

[00308] In a specific aspect, the clinical service form (CSF) of the antibody drug conjugates of the present disclosure is a lyophilisate in vial containing the ADC, sodium succinate, and polysorbate 20. The lyophilisate can be reconstitute with water for injection, the solution comprises the ADC, sodium succinate, sucrose, and polysorbate 20 at a pH of about 5.0. For subsequent intravenous administration, the obtained solution will usually be further diluted into a carrier solution.

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[00309] Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. In certain aspects, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, *e.g.*, Wawrzyniak, *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK, 1996; Kresina (ed.), *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, N.Y., 1991; Bach (ed.), *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, N.Y., 1993; Bacri *et al.*, *New Engl. J. Med.* 348:601-608, 2003; Milgrom *et al.*, *New Engl. J. Med.* 341:1966-1973, 1999; Slamon *et al.*, *New Engl. J. Med.* 344:783-792, 2001; Beniaminovitz *et al.*, *New Engl. J. Med.* 342:613-619, 2000; Ghosh *et al.*, *New Engl. J. Med.* 348:24-32, 2003; Lipsky *et al.*, *New Engl. J. Med.* 343:1594-1602, 2000).

[00310] Determination of the appropriate dose is made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.*, the inflammation or level of inflammatory cytokines produced.

[00311] Actual dosage levels of the active ingredients in the pharmaceutical compositions antibody drug conjugates can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors known in the medical arts.

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[00312] Compositions comprising antibodies or fragments thereof can be provided by continuous infusion, or by doses at intervals of, *e.g.*, one day, one week, or 1-7 times per week. Doses can be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, or by inhalation. A specific dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects.

[00313] For the immunoconjugates of the present disclosure, the dosage administered to a patient may be 0.0001 mg/kg to 100 mg/kg of the patient's body weight. The dosage may be between 0.0001 mg/kg and 20 mg/kg, 0.0001 mg/kg and 10 mg/kg, 0.0001 mg/kg and 5 mg/kg, 0.0001 and 2 mg/kg, 0.0001 and 1 mg/kg, 0.0001 mg/kg and 0.75 mg/kg, 0.0001 mg/kg and 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg or 0.01 to 0.10 mg/kg of the patient's body weight. The dosage of the antibodies or fragments thereof can be calculated using the patient's weight in kilograms (kg) multiplied by the dose to be administered in mg/kg.

[00314] Doses of the immunoconjugates can be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months. In a specific aspect, doses of the immunoconjugates of the present disclosure are repeated every 3 weeks.

[00315] An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method, route and dose of administration and the severity of side effects (see, *e.g.*, Maynard *et al.*, A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla., 1996; Dent, Good Laboratory and Good Clinical Practice, Urch Publ., London, UK, 2001).

[00316] The route of administration may be by, *e.g.*, topical or cutaneous application, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or by sustained release systems or an implant (see, *e.g.*, Sidman *et al.*, Biopolymers 22:547-556, 1983; Langer *et al.*, J. Biomed. Mater. Res. 15:167-277, 1981; Langer, Chem. Tech. 12:98-105, 1982; Epstein *et al.*, Proc. Natl. Acad. Sci. USA 82:3688-3692, 1985; Hwang *et al.*, Proc. Natl. Acad. Sci. USA 77:4030-4034, 1980; U.S. Pat. Nos. 6,350,466 and 6,316,024). Where necessary, the composition may also include a solubilizing agent or a local anesthetic such as lidocaine to ease pain at the site of the injection, or both. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

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See, *e.g.*, U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety.

[00317] A composition of the present disclosure can also be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Selected routes of administration for the immunoconjugates include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. Parenteral administration may represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, a composition of the present disclosure can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. In one aspect, the immunoconjugates of the present disclosure are administered by infusion. In another aspect, the immunoconjugates are administered subcutaneously.

[00318] If the immunoconjugates of the present disclosure are administered in a controlled release or sustained release system, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, CRC Crit. Rev Biomed. Eng. 14:20, 1987; Buchwald *et al.*, Surgery 88:507, 1980; Sandek *et al.*, N. Engl. J. Med. 321:574, 1989). Polymeric materials can be used to achieve controlled or sustained release of the therapies of the immunoconjugates (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla., 1974; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61, 1983; see also Levy *et al.*, Science 228:190, 1985; During *et al.*, Ann. Neurol. 25:351, 1989; Howard *et al.*, J. Neurosurg. 71:105, 1989; U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), poly glycolides (PLG),

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polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one aspect, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. A controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138, 1984).

[00319] Controlled release systems are discussed in the review by Langer, *Science* 249:1527-1533, 1990). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more immunoconjugates of the present disclosure. See, e.g., U.S. Pat. No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning *et al.*, *Radiotherapy & Oncology* 39:179-189, 1996; Song *et al.*, *PDA Journal of Pharmaceutical Science & Technology* 50:372-397, 1995; Cleek *et al.*, *Proc. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854, 1997; and Lam *et al.*, *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760, 1997, each of which is incorporated herein by reference in their entirety.

[00320] If the immunoconjugates of the disclosure are administered topically, they can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's *Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms*, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity, in some instances, greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, in some instances, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

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[00321] If the compositions comprising the immunoconjugates are administered intranasally, it can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present disclosure can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00322] Methods for co-administration or treatment with a second therapeutic agent, e.g., a cytokine, steroid, chemotherapeutic agent, antibiotic, or radiation, are known in the art (see, e.g., Hardman *et al.*, (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10.sup.th ed., McGraw-Hill, New York, N.Y.; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice: A Practical Approach, Lippincott, Williams & Wilkins, Phila., Pa.; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., Pa.). An effective amount of therapeutic may decrease the symptoms by at least 10%; by at least 20%; at least about 30%; at least 40%; or at least 50%.

[00323] Additional therapies (e.g., prophylactic or therapeutic agents), which can be administered in combination with the immunoconjugates may be administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours apart from the immunoconjugates of the present disclosure. The two or more therapies may be administered within one same patient visit.

[00324] In certain aspects, immunoconjugates can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds,

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To ensure that the therapeutic compounds of the disclosure cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.*, Ranade, (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Pat. No. 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (Bloeman *et al.*, (1995) *FEBS Lett.* 357:140; Owais *et al.*, (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe *et al.*, (1995) *Am. J. Physiol.* 1233:134); p 120 (Schreier *et al.*, (1994) *J. Biol. Chem.* 269:9090); see also K. Keisänen, M. L. Laukkanen (1994) *FEBS Lett.* 346:123; J. J. Killian; I. J. Fidler (1994) *Immunomethods* 4:273.

[00325] The present disclosure provides protocols for the administration of pharmaceutical composition comprising immunoconjugates alone or in combination with other therapies to a subject in need thereof. The combination therapies (*e.g.*, prophylactic or therapeutic agents) can be administered concomitantly or sequentially to a subject. The therapy (*e.g.*, prophylactic or therapeutic agents) of the combination therapies can also be cyclically administered. Cycling therapy involves the administration of a first therapy (*e.g.*, a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (*e.g.*, a second prophylactic or therapeutic agent) for a period of time and repeating this sequential administration, i.e., the cycle, in order to reduce the development of resistance to one of the therapies (*e.g.*, agents) to avoid or reduce the side effects of one of the therapies (*e.g.*, agents), and/or to improve, the efficacy of the therapies.

[00326] The therapies (*e.g.*, prophylactic or therapeutic agents) of the combination therapies of the disclosure can be administered to a subject concurrently.

[00327] The term "concurrently" is not limited to the administration of therapies (*e.g.*, prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising antibodies or fragments thereof are administered to a subject in a sequence and within a time interval such that the immunoconjugates can act together with the other therapy(ies) to provide an increased benefit than if they were administered otherwise. For example, each therapy may be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy

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can be administered to a subject separately, in any appropriate form and by any suitable route. In various aspects, the therapies (e.g., prophylactic or therapeutic agents) are administered to a subject less than 15 minutes, less than 30 minutes, less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, 24 hours apart, 48 hours apart, 72 hours apart, or 1 week apart. In other aspects, two or more therapies (e.g., prophylactic or therapeutic agents) are administered to a within the same patient visit.

[00328] The prophylactic or therapeutic agents of the combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

EXAMPLES

Example 1: Identification of CDH6 antibodies by phage-display technology

HuCAL PLATINUM® Pannings

[00329] Antibodies were identified by the selection of clones that bound to CDH6-ECD. As a source of antibody variants a commercially available phage display library, the Morphosys HuCAL PLATINUM® library, was used. The phagemid library is based on the HuCAL® concept (Knappik et al., J Mol Biol. 2000; 296(1):57-86; Prussler et al., J Mol Biol. 2011; 413(1):264-78 2011) and employs the CysDisplay™ technology for displaying the Fab on the phage surface (Rothe et al., J Mol Biol. 2008; 376(4):1182-200). For isolation of anti-CDH6 antibodies, standard panning strategies were performed using solid phase and solution panning approaches.

Overview of antigen (recombinant protein and cell lines) and antibody reagents used for pannings and screenings

[00330] Pannings were done on various forms of CDH6 antigens. An overview of recombinant CDH6 proteins and CDH6-expressing cell lines used as antigens for pannings and subsequent screening and characterization is given in Table 1.

[00331] Human, mouse and rat CDH6 extracellular domains were gene synthesized based on amino acid sequences from the GenBank or Uniprot databases (see Table 1). Cynomolgus CDH6

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cDNA template was gene synthesized based on amino acid sequences and homology information generated using mRNA from various cyno tissues (e.g. Zyagen Laboratories, San Diego, CA). All synthesized DNA fragments were cloned into appropriate expression vectors listed affinity tags to allow for purification or expression vectors Zyagen Laboratories for mammalian cell surface expression.

[00332] For the generation of a stable CHO cell line exogenously expressing human CDH6 the TREX expression system (Invitrogen, Carlsbad, CA) was used according to the manufacturer's instructions. Briefly, CHO-TREX cells (Invitrogen, R718-07, Carlsbad, CA) were grown in DMEM media (Invitrogen 11995-085) with 10% FBS (Invitrogen, 10082-147, Carlsbad, CA), and 10 µg/ml Blasticidin (Invitrogen A11139-02, Carlsbad, CA). Transfection was performed in 6 well plates when cell reach 90% confluence. 4 µg of linearized CDH6 plasmid DNA was mixed with 100 µl DMEM media (no FBS) in a sterile Eppendorf tube. 12 µl of Lipofectamine® 2000 (Invitrogen 11668-019, Carlsbad, CA) were mixed with 100 µl DMEM media in another sterile Eppendorf tube. DNA and Lipofectamine® were mixed together, and incubated in room temperature for 15 min. The culture media of CHO-TREX cells was changed to 1 ml DMEM media without FBS for each well; half above DNA/Lipofectamine® mix were added into each well, incubated for 2 hours. Then 2 ml growth media (with FBS) were added into each well, and the cells were incubated overnight. The transfected cells were split from 6 well plate to T175 flask next day and grew in selection DMEM media with 10% FBS, 10 µg/ml Blasticidin, 800-1000 µg/ml Geneticin (Invitrogen 10131-027). CDH6 expression was induced with final 1 µg/ml Tetracycline in selection media for 20-24 hours. Positive cells were labeled with final 5 µg/ml anti-CDH6 primary monoclonal antibody (MAB2715 from R&D Systems, Minneapolis, MN), and then labeled with PE conjugated anti-mouse secondary antibody (cat #12-4010-87, eBioscience, San Diego, CA) and sorted by FACS.

[00333] Stable CHO cell lines featuring exogenous expression of CDH6 from mouse, rat and cynomolgus origin were generated by transfection of CHO-K1 cells (Invitrogen, Carlsbad, CA) with the respective cDNAs cloned into a mammalian expression vector (pcDNA6.1, Invitrogen, Carlsbad, CA). Transfection was performed in 6 well plates when cell reach 90% confluence. 4 µg of linearized CDH6 plasmid DNA were mixed with 100 µl DMEM media without FBS in a sterile Eppendorf tube. 12 µl of Lipofectamine® 2000 (Invitrogen 11668-019, Carlsbad, CA) were mixed with 100 µl DMEM media in another sterile Eppendorf tube. The DNA and Lipofectamine® solution were mixed together, and incubated at room temperature for 15 min. The culture media of CHO cells was changed to 1 ml DMEM media without FBS for each well; half above DNA/Lipofectamine® mix were added into each well, incubated for 2 hours. Then 2 ml growth media (with FBS) were added into each well, and the cells were incubated overnight. The transfected cells were split from 6 well plate to T175 flask next day and grew in selection DMEM media with 10% FBS, 10 µg/ml Blasticidin, 800-1000 µg/ml Geneticin (Invitrogen 10131-027 Carlsbad, CA). CDH6 expression was induced with final 1 µg/ml

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Tetracycline in selection media for 20-24 hours. Positive cells were labeled with final 5 µg/ml anti-CDH6 primary monoclonal antibody (MAB2715 from R&D Systems, Minneapolis, MN), and then labeled with PE conjugated anti-mouse secondary antibody (cat #12-4010-87, eBioscience, San Diego, CA) and sorted by FACS. An alternative commercially available anti-CDH6 antibody is the 2B6 antibody (#GWB-E8FDF3 - Genway, San Diego, CA). This antibody was also used to confirm CDH6 expression in cells.

Table 1

Overview of antigen, antibody and cell line reagents for pannings and screenings

Antigen/Antibody	Accession/Source	Description	Amino acid sequence
His6-SUMO-CDH6 aa54-260-APP-Avi	NM_004932.3	human EC 1/2 domain variant (SEQ ID NO:3)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIHENTGD IQATKRLDREEKPVYILRAQAINRRRTGRP VEPESEFHKIHDINDNEPIFTKEVYTATVP EMSDVGTFVQVVTATDADDPTYGNSAK VVYSILQGGPYFSVESETGHIKTALLNMD RENREQYQVVIQAKDMGGQMGGLSGTT TVNITLTDGGGGSEFRHDSGLNDIFEAOKEWHE
hCDH6aa54-615 APP-Avi	NM_004932.3	human full-length ECD (SEQ ID NO:4)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIHENTGD IQATKRLDREEKPVYILRAQAINRRRTGRP VEPESEFHKIHDINDNEPIFTKEVYTATVP EMSDVGTFVQVVTATDADDPTYGNSAK VVYSILQGGPYFSVESETGHIKTALLNMD RENREQYQVVIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRPPOSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG LDMFDVITDQETQEGITVKLLDFEKKK VYTLKVEASNPYVEPRFLYLGPFKDSAT VRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETTFVCEKAKADQLIQLHAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRIHEMSTYLLFPVIS DNDYPVQSSFTGTVTVRVACDHHGNMQ SCHAEALIHPTGLSTGA
hCDH6aa54-615 APP-Avi-biotin	NM_004932.3	human full-length ECD, biotinylated (SEQ ID NO:5)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIHENTGD IQATKRLDREEKPVYILRAQAINRRRTGRP VEPESEFHKIHDINDNEPIFTKEVYTATVP EMSDVGTFVQVVTATDADDPTYGNSAK VVYSILQGGPYFSVESETGHIKTALLNMD RENREQYQVVIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRPPOSTYQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG

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			<p>LDMFDVITDQETQEGHITVKKLLDFEKKK VYTLKVEASNPYVEPRFLYLGPFKDSAT VRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFVCEKAKADQLIQTLHAVDK DDPYSGHQPSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPVVIS DNDYPVQSSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSEFRHDSGLND IFEAQK(BIOTIN)IEWHE</p>
hCDH6aa54-615 Fc	NM_004932.3	human full-length ECD, dimerized (SEQ ID NO:6)	<p>SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFINENTGD IQATKRLDREEKPVYILRAQAINRRRTGRP VEPESEFIKIHNDINDNEPIFTKEVYTATVP EMSDVGTFFVQVTATDADDPTYGNSAK VVYSILQGQPYFSVESETGHIKTALLNMD RENREQYQYVIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTVQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG LDMFDVITDQETQEGHITVKKLLDFEKKK VYTLKVEASNPYVEPRFLYLGPFKDSAT VRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFVCEKAKADQLIQTLHAVDK DDPYSGHQPSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPVVIS DNDYPVQSSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSDKTHTCPPCP APELLGGPSVFLFPPKPKDILMSRTP EVT CVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTHSKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLD DGSFELYSKLTVDKSRWQQGNVFCSVM HEALHNHYTQKSLSLSPGK</p>
cynoCDH6 FL	See above description on cynomolgus sequence derivation	Cynomolgus (<i>Macaca fascicularis</i>) full-length CDH6 (SEQ ID NO:7)	<p>SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFINENTGD IQATKRLDREEKPVYILRAQAINRRRTGRP VEPESEFIKIHNDINDNEPIFTKEVYTATVP EMSDVGTFFVQVTATDADDPTYGNSAK VVYSILQGQPYFSVESETGHIKTALLNMD RENREQYQYVIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTVQFKTPESSP PGTPIGRIKASDADVGENAEIEYSITDGEG LDMFDVITDQETQEGHITVKKLLDFEKKK VYTLKVEASNPVHVEPRFLYLGPFKDSAT VRIVVEDVDEPPVFSKLAYILQIREDAQIN TTIGSVTAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLLWHNIT</p>

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			VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFFVCEKAKADQLIQTILRAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPVVIS DNDYPVQSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSEFRHDSGLND IFEAQKIEWHE
moCDH6 FL	NM_007666.3	Mouse (<i>mus musculus</i>) full-length CDH6 (SEQ ID NO:8)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAVNRRRTGRP VEPESEFIKIHDINDNEPIFTKDVYTATVP EMADVGTFFVQVTAIDADDPTYGNSAK VVYSILQGGPYFSVESETGIKKTALLNMD RENREQYQVVIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRKASDADVGENAEIEYSITDGE HEMFDVITDQETQEGITVKKLLDFEKKK VYTLKVEASNPHVEPRFLYLGPFKDSAT VRIVVDDVDEPPVFSLAYILQIREDARIN TTIGSVAAQDPDAARNPVKYSVDRHTD MDRIFNIDSGNGSIFTSKLLDRETLWHNI TVIATEINNPKQSSRVPLYIKVLDVNDNA EFAEFYETFFVCEKAKADQLIQTILRAVD KDDPYSGHQFSFSLAPEAASSNFTIQDN KNTAGILTRKNGYNRHEMSTYLLPVVI SDNDYPVQSSTGTVTVRVCACDHHGNM QSCHAEALIHPTGLSTGAGSEFRHDSGLN DIFEAQKIEWHE
ratCDH6 FL	NM_012927.1	Rat (<i>Rattus norvegicus</i>) full-length CDH6 (SEQ ID NO:9)	SWMWNQFFLLEEYTGSDYQYVGKLHSD QDRGDGSLKYILSGDGAGDLFIINENTGD IQATKRLDREEKPVYILRAQAINRRRTGRP VEPESEFIKIHDINDNEPIFTKDVYTATVP EMADVGTFFVQVTAIDADDPTYGNSAK VVYSILQGGPYFSVESETGIKKTALLNMD RENREQYQVVIQAKDMGGQMGGLSGTT TVNITLTDVNDNPPRFPQSTYQFKTPESSP PGTPIGRKASDADVGENAEIEYSITDGE HDMFDVITDQETQEGITVKKLLDFEKKR VYTLKVEASNPHIEPRFLYLGPFKDSATV RIVVDDVDEPPVFSLAYILQIREDAQINT TIGSVAAQDPDAARNPVKYSVDRHTDM DRIFNIDSGNGSIFTSKLLDRETLWHNIT VIATEINNPKQSSRVPLYIKVLDVNDNAP EFAEFYETFFVCEKAKADQLIQTILHAVDK DDPYSGHQFSFSLAPEAASGSNFTIQDNK DNTAGILTRKNGYNRHEMSTYLLPVVIS DNDYPVQSSTGTVTVRVCACDHHGNMQ SCHAEALIHPTGLSTGAGSEFRHDSGLND IFEAQKIEWHE

Recombinant protein expression and purification:*His6-SUMO-CDH6 aa34-260-APP-Avi*

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[00334] Protein was expressed in Rosetta® 2 De3 pLysS cells (Millipore, Billerica, MA) in pET vector using 2xYT + 50 µg/ml Kanamycin and 30 µg/ml chloramphenicol. Cells were induced with 0.1 mM IPTG at 30°C. The pellet resuspended in 400 ml 20mM Tris-Cl, pH 8.0, 150 mM NaCl, 3mM CaCl₂, 20 mM Imidazole, + PI tabs (1 per 50 ml) + 1mg/ml lysozyme added fresh, homogenized to break up clumps and passed through microfluidizer 3 times. Following centrifugation at 20K x g for 30 min the soluble fraction of supernatant was used for purification over a 2 ml Ni-NTA (Qiagen superflow resin, Qiagen, Venlo, Netherlands). The lysate was passed over the column at 2.0 ml/min, washed with 10 CV (column volume) 20mM Tris-Cl, pH 8.0, 150 mM NaCl, 3mM CaCl₂, 20 mM Imidazole and protein was eluted with 4 x 3.0 ml elution fractions using 20mM Tris-Cl, pH 8.0, 500 mM NaCl, 3mM CaCl₂, 250 mM Imidazole. Fractions were pooled. 200 units of SUMO Protease (Invitrogen) were added with ~15ml protein (~20mgs) and dialyzed into 5L of 20 mM Tris-Cl, pH 8.0, 150 mM NaCl, 3 mM CaCl₂ overnight at 4°C using Snakeskin® pleated tubing with 10K cutoff (Thermo Scientific, Rockford, IL). Dialyzed material was collected and re-purified over 2.0 ml Ni-NTA resin to remove uncleaved protein and SUMO Protease. Cleaved protein was collected from flow through and wash fractions. Concentrated flow through and wash from reverse Ni-NTA to 5 ml volume, added NaCl to final concentration of 500mM and injected onto S200 column, buffer = 20mM Tris-Cl, pH 8.0, 150 mM NaCl, 3mM CaCl₂.

hCDH6aa54-615 APPavi, hCDH6aa54-615 Fc, cynoCDH6 FL, moCDH6 FL, ratCDH6 FL

[00335] The protein was expressed in HEK293 (ATCC CRL-1573) derived cell lines previously adapted to suspension culture and grown in a Novartis proprietary serum-free medium. Small scale expression verification was undertaken in transient 6-well-plate transfection assays on the basis of lipofection. Large-scale protein production via transient transfection and was performed at the 10-20 L scale in the Wave™ bioreactor system (GE Healthcare, Pittsburgh, PA). DNA Polyethylenimine (Polysciences, Warrington, PA) was used as a plasmid carrier at a ratio of 1:3 (w:w). The cell culture supernatants were harvested 7-10 days post transfection and concentrated by cross-flow filtration and diafiltration prior to purification.

[00336] For purification, clarified and filtered conditioned supernatant from the transient transfection was passed over a 20 ml affinity column at 5ml/min. Wash 20 CV PBS, 1% TX100, 0.3% t-n-butylphosphate buffer, 20CV PBS. Elute using 100 mM sodium citrate, 150 mM NaCl pH3.0, protein collecting 2 ml fractions. Pooled fractions were neutralized and then dialyzed overnight at 4°C with 5L 50 mM Tris, 150 mM NaCl pH 7.4, 3 mM CaCl₂. Sample was then subjected to size exclusion chromatography on a Superdex® S200 16/60 column (GE Healthcare, Pittsburgh, PA) equilibrated with dialysis buffer.

[00337] An overview of the various panning strategies is indicated in Table 2

Table 2

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Overview of panning strategies

Pancode	Phage subpool	Target and conditions
1038.1	VH1A/B/5 $\lambda+\kappa$	SP on hCDH6aa54-615 APPavi, without CaCl_2
1038.2	VH3 $\lambda+\kappa$	
1038.3	VH2/6 $\lambda+\kappa$	
1038.4	VH6, κ	
1038.5	VH1A/B/5 $\lambda+\kappa$	SP on hCDH6aa54-615 APPavi, with CaCl_2
1038.6	VH3 $\lambda+\kappa$	
1038.7	VH2/6 $\lambda+\kappa$	
1038.8	VH6, κ	
1038.9	VH1A/B/5 $\lambda+\kappa$	SP on His6-SUMO-CDH6 aa54-260-APP-Avi, with 1mM CaCl_2
1038.10	VH3 $\lambda+\kappa$	
1038.11	VH2/6 $\lambda+\kappa$	
1038.12	VH6, κ	
1038.13	VH1A/B/5 $\lambda+\kappa$	SP on His6-SUMO-CDH6 aa54-260-APP-Avi, with 1mM CaCl_2
1038.14	VH3 $\lambda+\kappa$	
1038.15	VH2/6 $\lambda+\kappa$	
1038.16	VH6, κ	
1038.17	VH1A/B/5 $\lambda+\kappa$	LP on biotinylated hCDH6aa54-615 APPavi
1038.18	VH3 $\lambda+\kappa$	
1038.19	VH2/6 $\lambda+\kappa$	
1038.20	VH1A/B/5 $\lambda+\kappa$	
1038.21	VH3 $\lambda+\kappa$	Pull-down panning using biotinylated tool antibody 2B6
1038.22	VH2/6 $\lambda+\kappa$	
1038.23	VH1A/B/5 $\lambda+\kappa$	LP on Biotinylated hCDH6aa54-615-Fc
1038.24	VH3 $\lambda+\kappa$	
1038.25	VH2/6 $\lambda+\kappa$	
1038.26	VH1A/B/5 $\lambda+\kappa$	SP on hCDH6aa54-615-Fc
1038.27	VH3 $\lambda+\kappa$	
1038.28	VH2/6 $\lambda+\kappa$	

Biotinylation of proteins

[00338] Avi-tagged proteins were biotinylated using BirA enzyme following manufacture (Avidity, Aurora, CO) standard protocol for protein biotinylation on an Avi-tagged protein. After

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biotinylation the protein was subjected to size exclusion chromatography on a Superdex® S200 16/60 column into 50 mM Tris, 150 mM NaCl pH 7.4, 3 mM CaCl₂.

[00339] Non-Avi-tagged proteins were dialyzed using Slide-A-Lyzer Mini Dialysis units, 10000 MWCO (#69576 Thermo Scientific, Rockford, IL) twice for 2h against 5L of 1x PBS under stirring at room temperature prior to biotinylation. EZ-link Sulfo-NHS-LC-biotin (#21327 Thermo Scientific, Rockford, IL) was reconstituted according to the manufacturer's protocol. The proteins were incubated with a 20-fold molar excess of biotin reagent for 30 min at room temperature. To remove non-reacted biotin reagent the sample was again dialyzed twice for 2h against 5L of 1x PBS under stirring at room temperature. Protein concentrations were determined by means of the Nanodrop® device (Thermo Scientific, Rockford, IL). Samples were stored at 4°C until usage.

Solid phase panning

[00340] In solid phase panning the Fab fragment displaying phages are incubated with antigen that has been bound to a surface support by direct immobilization.

[00341] Prior to the antigen selection process, a coating check ELISA was performed to determine the optimal antigen coating concentration. For this purpose a 2 fold dilution series of hCDH6aa54-615 APP_{avi} (see Table 1) covering 24 to 0.19 µg/mL was generated. The individual dilutions were used to coat wells on a 96-well Maxisorp™ plate (#442404 Nunc, Rochester, NY) via direct immobilization. After coating the plate was washed thrice with 300 µL PBS and subsequently blocked with 1x blocking buffer (2.5% milk, 2.5% BSA, 0.05% Tween 20) for 2h at room temperature.

[00342] To detect bound antigen, an anti-APP antibody (generated in-house) was added to 1 µM, 0.18 µM or 0.04 µM. Secondary detection was done using AP-labeled anti-mouse IgG-F(ab)₂ specific antibody in a 1:5000 dilution Ref. 115-056-006 Jackson-ImmunoResearch, West Grove, PA). The antigen concentration at which signal saturation was observed was chosen as coating concentrations for SP panning.

Pannings 1038.1-8

[00343] For SP panning 1038.1-8 (see Table 2) hCDH6aa54-615 APP_{avi} (see Table 1, SEQ ID NO:4) was coated on a 96-well Maxisorp™ plate (Nunc) via direct immobilization. Two wells were coated with 300 µL antigen solution (10 µg/mL in PBS) per phage subpool combination. Coating was done at 4°C overnight with (pannings 1038.1-4) or without (pannings 1038.4-8) the presence of 1 mM CaCl₂. The wells were subsequently washed twice with 400 µL PBS and blocked with 400 µL blocking buffer for 2h at room temperature at 400 rpm. After blocking the cells were washed twice with 400 µL PBS.

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[00344] Prior to binding phage subpools were blocked and depleted with unrelated FLT2-APPavi at 10 µg/mL for 1h at room temperature to eliminate unspecific and tag binders. 300 µL of blocked and depleted phage pools were transferred per coated well and incubated for 2h at room temperature and 400 rpm. Non-specific bound phages were removed by the washing steps listed below in Table 3.

Table 3

First Round	Second Round	Third round (only panocodes 1038.9-16)
3x PBST (quick)	1x PBST (quick)	10x PBST (quick)
2x PBST / 5min	4x PBST / 5min	5x PBST / 5min
3x PBS (quick)	1x PBS (quick)	10x PBS (quick)
2x PBS / 5min	4x PBS / 5min	5x PBS / 5min

[00345] The specifically bound phages were eluted with 300 µL elution buffer for 10 min at room temperature. Eluted phages were used to reinfect 14 mL of E. coli TGIF+ (Stratagene/Agilent, Santa Clara, CA) at an optical density of OD₆₀₀=0.6-0.8. The mix of E. coli TGIF+ and phage eluate was incubated for 45 min in a water bath at 37°C for phage infection. The bacterial pellets were re-suspended in 2xYT medium, plated on LB agar plates supplemented with 34 µg/mL chloramphenicol and incubated overnight at 37°C. Colonies were scraped off the plates and were used for polyclonal amplification of enriched clones and phage production. With purified phage the next panning round was started. The second round of pannings was performed according to the protocol of the first round except for a more stringent washing condition. A third panning round was omitted due to the low second round output titers of ~10⁴ cfu/mL.

Panocodes 1038.9-16

[00346] For SP pannings 1038.9-16 (Table 2) His6-SUMO-CDH6 aa54-260-APP-Avi (see Table 1) was coated on a 96-well Maxisorp™ plate (Nunc) via direct immobilization as described for panocodes 1038.1-8. For pannings 1038.13-16 300 µL of a 100 nM solution of tool antibody MAB2715 was added per well and incubated for 1 h at room temperature to avoid selection of binders recognizing the same epitope as MAB2715. Besides the antigen coated and a third panning round the pannings were done as described for panocodes 1038.1-8.

Panocodes 1038.26-28

[00347] For SP pannings 1038.26-28 (see Table 2) hCDH6aa54-615-Fc (see Table 1, SEQ ID NO.6) was coated on a 96-well Maxisorp™ plate (Nunc) via direct immobilization as described for panocodes 1038.1-8. Besides the antigen coated and the washing stringencies the pannings were done as described for panocodes 1038.1-8.

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Liquid phase panning

[00348] During solution panning, the Fab fragment displaying phage and biotinylated antigens are incubated in solution which is expected to increase accessibility of the antigen by the phage. Antigen biotinylation was done as described above.

Pancodes 1038.17-22

[00349] Each phage library subpool was blocked with an equal volume of 2x Chemblocker for 2h at room temperature on a turning wheel. To avoid selection of antibodies against the APPavi and biotin tag unrelated FLT3-APPavi and biotinylated anti-Cyclosporin mIgG was added to 7.5 and 18 µg/mL in the blocking step, respectively. For removal of phage particles binding to Streptavidin-beads, pre-adsorption of blocked phage particles was performed using blocked Streptavidin beads (# 112.06D Invitrogen, Carlsbad, CA). Blocked and pre-adsorbed phages were incubated with biotinylated hCDH6aa54-615 APPavi (see Table 1, SEQ ID NO:4) for 1h at room temperature on a turning wheel. Phage-antigen complexes were captured either with washed beads (Pancodes 1038.17-19) or with beads precoated with biotinylated anti-CDH6 antibody 2B6 (Genway, San Diego, CA) to enrich binders for non-2B6 epitopes (Pancodes 1038.20-22). Non-specific phage particles were removed by several washing steps (see below). Elution of specifically bound phage particles, infection, and amplification were done as described for pancodes 1038.1-8.

Table 4

Washing conditions Pancodes 1038.17-25

First Round	Second Round	Third round (only pancodes 1038.9-16)
5x PBST (quick)	10x PBST (quick)	10x PBST (quick)
2x PBST / 5min	3x PBST / 5min	5x PBST / 5min
3x PBS (quick)	5x PBS (quick)	5x PBS (quick)

Pancodes 1038.23-25

[00350] Pannings were done as described for pancodes 1038.1-8 but using biotinylated hCDH6aa54-615-Fc (see Table 1, SEQ ID NO:6) as antigen.

Subcloning

Conversion for FAB-FH expression

[00351] To facilitate rapid expression of soluble Fab fragments, the Fab encoding inserts of the selected HuCAL® PLATINUM phage particles were subcloned from the pMORPH®30 display vector into the pMORPH®11_FH expression vector. Glycerol stocks containing E. coli that were

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infected with the final panning round output phages were used to inoculate cultures for pMORPH®30 DNA purification using the Nucleobond® Xtra Midi Plus kit according to the manufacturers manual (#740412.50 Machery Nagel, Bethlehem, PA). 5 µg of each pMORPH®30 DNA output pool was triple digested via EcoRI/XbaI/BmtI (all restriction enzymes were purchased from New England Biolabs, Ipswich, MA). The resulting EcoRI/XbaI Fab encoding 1485 bp fragment was gel purified using the Wizard SV Gel/PCR clean-up kit according to the manufacturers manual (A9282, Promega, Madison, WI) and ligated into the EcoRI/XbaI cut pMORPH®11_FH vector backbone.

Conversion for IgG expression

[00352] In order to express full length IgG, variable domain fragments of VH and VL were subcloned from Fab expression vectors into pMORPH®4_hlgG1F vectors following the RapCLONE® protocol (Morphosys, Martinsried/Planegg, Germany). RapCLONE® is a two-step cloning method for the batch conversion of a large amount of Fab expression vectors into IgG expression vectors. In a first cloning step, a eukaryotic expression cassette was introduced into the pMORPH®11 expression vectors via BsiWI/MfeI (for κ pools) or HpaI/MfeI (for λ pools) digestion and subsequent ligation. This was followed by a second cloning step, in which the Fab pools containing the expression cassette were digested using EcoRV/BlnI (both κ and λ pools) and subsequently cloned into the pMORPH®4_hlgG1F acceptor vector for expression in mammalian cells.

Expression and Purification of Fab Fragments

Transformation of E. coli TG1F

Electroporation-competent E. coli TG1F- aliquots of 50 µL were thawed on ice, mixed with 50 µg/µl pMORPH®11_FH plasmid DNA and transferred into pre-cooled electroporation cuvettes. The cells were electroporated (Bio-Rad Gene Pulser; settings: 1.75 kV, 200 Ω, and 25 µF (Bio-Rad, Hercules, CA), transferred into 950 µl pre-warmed SOB medium and incubated for 1 h at 37 °C shaking at 220 rpm. An appropriate volume of the transformation samples were plated onto LB agar plates supplemented with 34 µg/mL Chloramphenicol and incubated overnight at 37°C to obtain single colonies.

Generation of Master plates

[00353] Chloramphenicol resistant single clones were picked into the wells of a sterile 384-well microtiter plate (Nunc) pre-filled with 60 µl 2xYT medium supplemented with 34 µg/ml of Chloramphenicol and 1 % glucose and grown overnight at 37°C. Next morning, 20 µl sterile 2xYT media containing 60 % glycerol and 1 % glucose were added into each well of the master plates. Plates were sealed with aluminum foil and stored at -8 °C.

Generation of Fab-Containing Bacterial Lysates for ELISA Screening

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[00354] 5 μ l of each well of a Master plate was transferred to a sterile 384-well microtiter plate pre-filled with 40 μ l 2xYT medium per well supplemented with 34 μ g/ml of Chloramphenicol and 0.1 % glucose. Plates were incubated at 37°C at 480 rpm and 80% humidity until the cultures were slightly turbid. 10 μ l of 2xYT medium supplemented with 34 μ g/ml Chloramphenicol and 5 mM IPTG were added per well for induction of Fab fragment expression. Plates were sealed with a gas-permeable tape and incubated overnight at 22°C at 500 rpm and 80% humidity. The next day 15 μ l BEL lysis buffer (2.5 mg/ml lysozyme (#10837059001, Roche, Nutley, NJ), 4 mM EDTA, 10 U/ μ l Benzonase (# 1.01654.0001 Merck, White House Station, NJ) was added to each well and plates were incubated for 1 h at 500 rpm and 80% humidity. Resulting Fab lysates were blocked by adding 15 μ l of 2x Chemiblocker per well followed by incubation for 30 min at 22°C, 500 rpm and 80% humidity.

Generation of Fab-Containing Bacterial Lysates for FACS Screening

[00355] 5 μ l of each well of a compression plate was transferred to a sterile 96-well round bottom microtiter plate pre-filled with 100 μ l 2xYT medium per well supplemented with 34 μ g/ml of Chloramphenicol. Plates were incubated at 37°C at 500 rpm and 80% humidity until the cultures were slightly turbid. 10 μ l of 2xYT medium supplemented with 34 μ g/ml Chloramphenicol and 5 mM IPTG were added per well for induction of Fab fragment expression. Plates were sealed with a gas-permeable tape and incubated overnight at 22°C at 500 rpm and 80% humidity. The next day the plate was centrifuged for 10 min at 1200g and the supernatant discarded. The bacterial pellets were frozen overnight at -20°C to facilitate the lysis step. The thawed pellets were re-suspended in 200 μ l of BEL lysis buffer (see above) and incubated for 1 h at 22°C and 250 rpm. Resulting Fab lysates were centrifuged to remove cellular debris for 10 min at 1200 g. Fab containing supernatants were used for screening purposes.

Expression and Purification of His6-tagged Fab Fragments in E. coli

[00356] E. coli TG1F⁺ transformants containing pMORPH#11 Fab FH DNA were singled out on 2xYT supplemented with 34 μ g/mL Chloramphenicol and 1% glucose. Individual clones were picked and transferred into 3 mL 2xYT seed cultures supplemented with 34 μ g/mL Chloramphenicol and 1% glucose and incubated at 37°C, 220 rpm for 4h. The seed culture was used to inoculate 50 mL main cultures with 2xYT supplemented with 34 μ g/mL Chloramphenicol and 1% glucose and incubated in 250 mL shake flasks at 30°C until the OD600 reached a value of 0.6. Fab expression was induced by addition of IPTG to a final concentration of 0.75 mM and cultures were further incubated overnight at 25°C and 220 rpm. The next day cells were harvested and the cell pellets frozen overnight at -20°C. Cells pellets were disrupted by re-suspending and incubating in lysis buffer (25 mM TRIS/pH=8, 500 mM NaCl, 2 mM MgCl₂, 10 U/ μ l Benzonase (# 1.01654.0001 Merck), 0.1% Lysozyme (# 10837059001, Roche), Protease Inhibitor Complete w/o EDTA 1 tablet/ 50 mL of buffer (#11873580001 Roche)) for 1h at room temperature on a rocking table. Lysates were clarified by

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centrifugation for 30 min at 15000 g and filtration of the supernatant through a 200 nm pore sized filter. His6-tagged Fab fragments were isolated via immobilized metal ion affinity chromatography (Ni-NTA Superflow® beads, #30430 Qiagen, Venlo, Netherlands) and eluted using imidazole. Buffer exchange to 1x PBS was performed using PD-10 columns (# 17-0851-01 GE Healthcare, Pittsburgh, PA). Samples were sterile-filtered and the protein concentrations were determined by UV-spectrophotometry. The purity of the samples was analyzed in denaturing, reducing 15% SDS-PAGE. The identity of the samples was confirmed by MS.

Expression and purification of IgGs

Expression of IgGs at screening scale

[00357] Eukaryotic HEK293c18 cells (ATCC CRL-10852) were used in a 96-well expression system for the generation of conditioned cell culture supernatants containing full-length IgG for the subsequent use in specificity and/or functional assays. Eukaryotic HEK293 c18 cells were re-suspended in transfection medium (D-MEM supplemented with 2% L-glutamine (#25030-024 Gibco, Grand Island, NY) 10 % FCS (#3302 PAN Biotech, Aidenbach, Germany) and 1 % penicillin/streptomycin (#15140-122 Gibco, Grand Island, NY)) and seeded in 96-well F-bottom plates to a density of approximately 4×10^4 cells in 50 μ l per well the day before. A transfection master mix was prepared by mixing 0.6 μ l Lipofectamine® 2000 (#11668 Invitrogen, Carlsbad, CA) per well with 25 μ l Opti-MEM® I medium, (#31985-047 Invitrogen, Carlsbad, CA). The master mix was incubated for 15 min at room temperature. On a new plate 20 μ l of Opti-MEM® I medium was added to wells containing 300 ng DNA and gently mixed. After that, the DNA was combined with the pre-incubated Lipofectamine® 2000, mixed gently and incubated for 20 min at room temperature. 100 μ l of the pre-incubated Lipofectamine® 2000/DNA complexes were then transferred to each well of the plates with the cells and gently mixed. Plates were incubated for 40 h at 37°C and 6 % CO₂ for transient expression. The culture supernatants were transferred to 96-well V-bottom plates and cleared by centrifugation. The resulting IgG-containing supernatants were tested by an anti-Fd capture ELISA for assessment of IgG protein concentration in reference to a known standard and stored at -80°C for later use.

IgG expression check by anti-Fd ELISA

[00358] To assess IgG levels in screening scale derived supernatants Maxisorp ® 96-well plates (#437111 Nunc) were coated with 50 μ l/well Fd-fragment-specific sheep anti-human IgG (#PC075 The Binding Site, San Diego, CA) diluted 1:1000 in PBS. After coating, the plates were washed twice with TBST and blocked for 1h with 5 % skim milk powder in TBST. In the meantime the IgG containing supernatants were diluted 1:50 in 2.5% skim milk powder in TBST. After washing the ELISA plates three times with TBST, 100 μ l of the diluted supernatants were transferred per well and plates were incubated for 1 h at room temperature. Subsequently, the plates were washed five

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times with TBST and the captured IgGs were detected by incubation with 50 μ l F(ab')₂-specific goat anti-human IgG (#109-055-097 Dianova, Hamburg, Germany) (diluted 1:5000) in 0.5 % skim milk powder in TBST for 1h. After washing the plates five times with TBST the AttoPhos fluorescence substrate (#1484281 Roche, Nutley, NJ) was added according to the manufacturer's instructions and the fluorescence emission at 535 nm was recorded with excitation at 430 nm with an ELISA reader.

Expression and Purification of IgGs in Microscale

[00359] CAP-T® cells (CEVEC Pharmaceuticals, Cologne, Germany) were transiently transfected with pMORPH®4 IgG expression plasmid in FreeStyle293® expression medium (#12338 Invitrogen, Carlsbad, CA) using 40 kDa linear PEI (PEI Max (#24765-2 Polysciences Warrington, PA)) as gene delivery vehicle.

[00360] One day prior to the transfection cells were diluted to $\sim 0.8 \times 10^6$ cells/ml to ensure exponential growth. At the day of transfection cells were diluted in 9 mL pre-warmed Freestyle® (Invitrogen, Carlsbad, CA) medium to $\sim 0.5 \times 10^7$ cells/ml and transferred into a 125ml shake flask. 30 μ g DNA was diluted in 500 μ l OptiMEM. 1200 μ g PEI Max (#24765-2 Polysciences Warrington, PA) was diluted in 8.80 mL OptiMEM medium. The DNA solution was added drop wise to the cells and gently mixed. After that, 500 μ l PEI Max solution was added to the cells and gently mixed.

[00361] The cells were incubated at 37°C, 6 % CO₂, 85 % humidity with agitation at 100 rpm. After 4 h 10 mL pre-warmed PEM supplemented with 4 mM L-Glutamine and 5 μ g/ml blasticidin (#R21001 Invitrogen, Carlsbad, CA) was added. Simultaneously, 400 μ l valproic acid (#P4543-25G Sigma Aldrich, St. Louis, MO) was added to a final concentration of 4 mM. Cells were incubated for 6 days at 37°C, 6 % CO₂, 85 % humidity with agitation at 100 rpm.

[00362] After centrifugation for 5 min at 1200 g at 4°C the supernatant was sterile-filtered (0.2 μ m pore size) and subjected to a Protein A affinity chromatography (MabSelect® SURE, GE Healthcare, Pittsburgh, PA) using a liquid handling station. Buffer exchange was performed to 1x PBS and samples were sterile filtered (0.2 μ m pore size). Protein concentrations were determined by UV spectrophotometry and purity of IgGs was analyzed under denaturing, reducing conditions in SDS-PAGE.

Screening of Fab-containing raw bacterial lysates

ELISA screening

[00363] Using ELISA screening, single Fab clones were identified from panning outputs for binding to the target antigen. Fab fragments were tested using Fab containing crude E. coli lysates.

ELISA screening on directly coated antigen

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[00364] Maxisorp® (#442404 Nunc, Rochester, NY) 384-well plates were coated overnight at 4°C with huCDH6 proteins at a concentration of 3 µg/ml in PBS. After washing plates were blocked for 2 h with 5% skimmed milk in 1xPBST. Fab-containing E. coli lysates were added and binding allowed for 1 h at room temperature.

[00365] To detect bound Fab fragments plates were washed 5x with TBST and AP-anti human IgG F(ab')₂ (#109-055-097 Jackson ImmunoResearch, West Grove, PA) was added in a 1/2500 dilution. After 1h at room temperature plates were washed 5x with TBST and AttoPhos substrate was added according to the manufacturer's specifications. Plates were read in an ELISA reader 5 minutes after adding the substrate.

ELISA Screening on Biotinylated Antigen using NeutrAvidin® Plates

[00366] Maxisorp® (#442404 Nunc, Rochester, NY) 384-well plates were coated overnight at 4°C with NeutrAvidin® (#31000 Thermo Scientific, Rockford, IL) at a concentration of 10 µg/ml in PBS. After washing, plates were blocked for 2 h with 1x Chemiblocker. Fab-containing E. coli lysates were dispensed into a fresh 384-well microtiter plate and biotinylated CDH6 antigens added at a concentration of 2.5 µg/mL. Fab-antigen complexes were then transferred to the NeutrAvidin® coated plates and capturing allowed for 1h at room temperature and detected as described above.

FACS screening

[00367] In FACS screening, Fab fragments binding to cell surface expressed CDH6 antigen were identified from the panning output. 1×10^5 cells/well were transferred into U bottom 96 well plates and mixed with 40 µl/well of the Fab-containing bacterial lysates. Plates were incubated shaking at 4°C for 1 h. After the incubation, 100 µl/well ice-cold FACS buffer (3% FCS, 0.02% NaN₃, 2 mM EDTA in PBS) was added, cells were spun down at 4°C for 5 min at 250 g and washed twice with 180 µl/well ice-cold FACS buffer. After each washing step, cells were centrifuged and carefully re-suspended. After the last washing step cells were re-suspended in 50 µL of 1/200 diluted secondary detection antibody (PE-conjugated goat anti-human IgG (#109-116-088, Jackson ImmunoResearch, West Grove, PA). After 1h incubation at 4°C cells were again washed twice in 180 µL/well ice-cold FACS buffer. Finally, cell pellets were re-suspended in 120 µl/well FACS buffer with 0.4 % paraformaldehyde and analyzed in a FACSCalibur® (BD Biosciences, San Jose, CA) equipped with an HTS plate reader.

Clone sequencing

Fab clone sequencing

[00368] Confirmed cell binding Fab hits were subjected to VL and VH sequencing. 1.2 mL 2xYT supplemented with 34 µg/mL Chloramphenicol and 1% glucose were inoculated with 5 µL of bacterial glycerol stock deriving from the compression plates and grown in 96-well deep well

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microtiter plates overnight at 37°C and 500 rpm. The next day the bacteria were harvested and the pMORPH[®]11 FH plasmids purified using a 96-well DNA purification kit (Nucleospin[®] 96 Plasmid Purification Kit #740625.24, Machery Nagel, Bethlehem, PA) according to the manufacturers protocol. To sequence the VL and VH primers M13rev (5' CAGGAAACAGCTATGAC 3' (SEQ ID NO:10) and HnCAL_VH_for ((5' GATAAGCATGCGTAGGAGAAA 3 (SEQ ID NO:11)) were used, respectively.

IgG clone sequencing

[00369] Unique cell binding Fab clones were converted in the IgG1 format in a polyclonal manner and afterwards retrieved by VL and VH sequencing. 1.5 mL 2xYT medium supplemented with 100 µg/mL Amp and 1% glucose were inoculated with single clones and grown in 96-well deep well microtiter plates overnight at 37°C, 450 rpm and 80% humidity. The next day the bacteria were harvested and the IgG encoding pMORPH[®]4 plasmids purified using a 96-well DNA purification kit according to the manufacturer's protocol (Nucleospin[®] 96 Plasmid Purification Kit #740625.24, Machery Nagel, Bethlehem, PA). To sequence the VL and VH primers ((T7 5' TAATACGACTCACTATAGGG 3' (SEQ ID NO:12) and CMV HC for (5' CTCTAGCGCCACCATGAAACA 3' (SEQ ID NO:13)) were used, respectively.

[00370] *In vitro assays*

Affinity assessment using Octet[®] QK

[00371] Affinity assessments by determining kinetic parameters were performed via Bio-Layer Interferometry technology. All Fab samples were measured using Streptavidin Dip and Read biosensors (#18-0009 ForteBio, Menlo Park, CA). The plate was placed in an Octet[®] QK instrument (ForteBio, Menlo Park, CA) and allowed to equilibrate to 27°C in the chamber. The run was initiated by placing the sensors in the wells containing 150 nM of biotinylated CDH6 protein for 300s. After that the sensors were placed in the wells containing 250 nM Fab sample. Fab association and dissociation were each recorded for 600s by measuring the change in layer thickness (in nanometers, nm) with time, all under computer control. Data were processed automatically using the Octet[®] User Software version 3.0.

Assessment of antibody cellular internalization propensity

[00372] Cell Internalization of IgGs by target mediated endocytosis was assessed by microscopy using a VTI ArrayScan[®] HC reader (ThermoFischer, Waltham, MA) as described below. The underlying analysis protocol was the Spot Detector V4 algorithm (ThermoScientific Cellomics[®], Thermo Scientific, Rockford, IL). In brief, this analysis protocol provides a fast and generic spot analysis that identifies intracellular punctuate objects, such as IgG containing lysosomal vesicles after

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immunofluorescent staining. The total punctate fluorescent intensity can then be averaged over the number of cells analyzed.

[00373] OVCAR3 cells (ATCC HTB-161) were grown in T150 tissue culture flasks to approximately 90% confluency. Media was discarded and cells flushed with 10 mL PBS. Cells were detached by incubation with 4 mL cell dissociation buffer for 5 min at 37°C. Detached cells were re-suspended thoroughly in growth medium and transferred to a 50 mL tube. After counting the cells on a Vi-Cell® analyzer (Beckman Coulter, Brea, CA) the suspension was adjusted to 10^5 cells/mL in growth medium. 100 μ L of the cell suspension was seeded per well of a 96-well microtiter plate with transparent bottom. Plates were incubated for 24 h at 37°C in 5% CO₂ to allow the cells to adhere and to expand.

[00374] IgG containing cell culture supernatants were generated as described and diluted in PBS in a fourfold dilution series covering dilutions ranging from 1:4 to 1:128. 100 μ L of each sample dilution was dispensed per well of the cell containing microtiter plate and incubated for 2h at 37°C to permit IgG internalization. Subsequently, cells were fixed by adding 100 μ L 1x CellFix® reagent (#340181 BD Biosciences, San Jose, CA) per well. After 10 minutes plates were washed twice with PBS and cells then permeabilized by adding 100 μ L 0.1% Triton X-100 per well. After 10 min plates were washed again twice with PBS and blocked with 100 μ L 1x Odyssey blocking buffer (#927-40000 LiCor, Lincoln, NE) for 2 hours at room temperature. To detect internalized IgGs 100 μ L of a 1:100 dilution of secondary antibody Alexa Fluor® 488 goat anti-human IgG (#11001 Molecular Probes, Grand Island, NY) supplemented with a 1:10000 dilution of Hoechst nucleus staining reagent (#B2261 Sigma, St. Louis, Mo) were added per well and incubated for 1 hours at room temperature in the dark. Cells were then washed twice with PBS without final aspiration. Plates were then loaded into the Cellomics® VTI ArrayScan HC reader (ThermoFischer, Waltham, MA) and analyzed. The extent of IgG internalization was assessed by the mean average spot intensity (MSAI) per cell.

Surrogate ADC assay using anti-human Fab-DM1 conjugate

[00375] To test the ability of CDH6 antibodies to internalize after receptor binding and deliver cytotoxic payload, a surrogate ADC assay was performed mixing anti-human Fab-DM1 reagent (AffiniPure® Fab Fragment Goat Anti-Human IgG H+L conjugated with SMCC-DM1) with purified IgGs at a fixed 1:2 ratio. Cytotoxic potential was tested on the cancer cell line OVCAR3 (ovarian serous carcinoma, cultured in McCoy's + 20% FCS) as these cells show high expression of CDH6.

[00376] Cells in culture were counted and diluted in medium to a concentration of 1×10^5 cells/mL. 1000 cells/well were transferred to 384-well plates (Corning Costar#3707, Corning, Tewksbury, MA). Primary antibody/Fab-DM1 solution was prepared in 1.4mL Matrix tubes (Thermo, # 3790, Rockford, IL.) by combining 666nM Fab-DM1 with 333nM primary human IgG diluted in cell

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culture media and incubated at 37°C for 30 minutes. A 10-point, 1:3 serial dilution was prepared in a 384-well deep-well plate (Brandtech Scientific Inc #701355, Essex, CT) and 25µl were transferred per assay plate (triplicates) to yield a highest starting concentration of FAB-DM1/human IgG of 66nM and 33nM, respectively. For controls, wells with cells only (=100% viability control) and cells only incubated with Fab-DM1 (to check for unspecific killing of the secondary reagent) were prepared. Plates were incubated for 120 h at 37°C and 5% CO2. Cellular activity of the primary antibody/Fab-DM1 complexes was determined using CellTiter-Glo® reagent (#G7571 Promega, Madison, WI) according to the manufacturer's instructions. Viability was normalized to the cells only control.

Antibody screening summary

[00377] The HuCAL® PLATINUM phagemid library was used to select specific Fab fragments against human CDH6-ECD antigens. Recombinant human APPA_{vi} and Fc fusion as well as truncated domain APPA_{vi} fusion proteins were used for the pannings. HuCAL® PLATINUM antibody-phage particles were subjected in different subpool combinations to a total of 8 different panning strategies resulting in 28 panning output pools. In summary, six out of eight strategies have been productive and resulted in 771 ELISA positive screening hits.

[00378] FACS screening on CHO cells expressing human CDH6 as well as OVCAR3 cells resulted in 271 confirmed cell binding hits. To consolidate these cell binding hits VL and VH sequencing was performed leading to the identification of 53 unique Fab antibody clones.

[00379] To allow for the functional characterization, the Fab antibody clones were converted into the IgG format, yielding 47 unique IgG clones of which 44 were successfully expressed at screening scale. The purified, unique IgGs were subjected to a series of characterization assays including human/cyno/rat/mouse cross-reactivity by FACS, affinity ranking by Octet, cellular internalization assays as well as surrogate ADC assays using an anti-human IgG Fab-SMCC-DM1 secondary reagent. Based on these assays, human IgGs were selected for scaled up production, subsequent direct conjugation to ADC linker/payloads and testing as ADCs in *in vitro* and *in vivo* experiments.

[00380] The sequence information for the anti-CDH6 IgGs selected for further in-depth characterization are shown in Table 5.

Table 5

Sequence information of the IgGs selected for in-depth characterization.

NOV ID	Category	Value
NOV0670	LCDR1	SGSSSNIGSQYVY

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(Kabat) (SEQ ID NO: 14)	
LCDR2 (Kabat) (SEQ ID NO: 15)	YNSERPS
LCDR3 (Kabat) (SEQ ID NO: 16)	QTWDASSQS FV
HCDR1 (Kabat) (SEQ ID NO: 17)	SYAIS
HCDR2 (Kabat) (SEQ ID NO: 18)	GHIPIFGTANYAQKFQG
HCDR3 (Kabat) (SEQ ID NO: 19)	KFPGRGPFAY
vH full sequence (SEQ ID NO: 20)	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPG QGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSL RSED TAVYYCARKFPGRGPFAYWGQGTLVTVSS
vL full sequence (SEQ ID NO: 21)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSQYVYWYQQLPGTA PKLLIYNSERPSGMFDRFSGSKSGTSASLAITGLQAEDEADYY CQTWDASSQS FVFGGGTKLTVL
vH DNA sequence (SEQ ID NO: 22)	CAGGTGCAATTGGTGACAGAGCGGTGCCGAAGTGAAAAAAC CGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCGGAGG GACGTTTCTTCTTACGCTATCTCTTGGGTGCGCCAGGCCCC GGGCCAGGGCCTCGAGTGGATGGGCGGTATCATCCCGATCT TCGGCACTGCGAACTACGCCCAGAAATTTCAGGGCCGGGTG ACCATTAACCGCGATGAAAGCACCAGCACCGCCTATATGGA ACTGAGCAGCCTGCGCAGCGAAGATACGGCCGTGTATTATT GCGCGCGTAAATTCCCGGGTCTGTGGTCCGTTCGCTTACTGGG GCCAAGGCACCCYGGTGACTGTAGCTCA
vL DNA sequence (SEQ ID NO: 23)	GATATCGTGCTGACCCAGCCGCGCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTCAGTACGTGTACTGGTACCAGCAGCTGCCG GGCACGGCGCCGAACTGCTGATCTACTACAACTCTGAACG CCCGAGCGGCATGCCGGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGACTTGGGACGCTTCTTCT CAGTCTTCTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
HC full sequence (SEQ ID NO: 24)	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPG QGLEWMGGIPIFGTANYAQKFQGRVTITADESTSTAYMELSSL RSED TAVYYCARKFPGRGPFAYWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSQLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKR

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		VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:25)	DIVLTQPPSVSGAPQRVTISCSGSSSNIGSQYVYVYQQLPGTA PKLLIYNSERPSGMPDRFSGSKSOTASLAIITGLQAEDEADYY CQTWDASSQSFVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN KYAASSYLSLTPEQWKSHRSYSQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:26)	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAAAC CGGGCAGCAGCGTGAAAGTTAGCTGCAAAGCATCCGGAGG GACGTTTCTTCTTACGCTATCTTGGGTGCGCCAGGCCCC GGGCCAGGGCCTCGAGTGGATGGGCGGTATCATCCCGATCT TCGGCACTGCGAACTACGCCCAGAAATTCAGGGCCGGGTG ACCATTACCGCCGATGAAAGCACCAGCACCCTATATGGA ACTGAGCAGCCTGCGCAGCGAAGATACGGCCGTGTATTATT GCGCGCGTAAATTCGGGGTCTGTTGCTCCGTTCTGCTTACTGG GCCAAGGCACCTGGTGACTGTTAGCTCAGCCTCCACCAAG GGTCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTA CTTCCCCGAACCGGTGACGGTGTCTGTGAACTCAGCGGCC TGACCAGCGGGGTGCACACCTTCCCGGCTGTCTACAGTCT CAGGACTTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACCTACACATGCCACCGTGCCAGC ACCTGAACTCTTGGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCTCATGATCTCCCGAACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAGC TACCGGGTGGTACGCTCTCACCCTCTGCACCAAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACATAAGAACACCG CCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGC AAGCTCACCCTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:27)	GATATCGTGCTGACCCAGCCGCGGAGCGTGAGCGGTGCACC GGGCCAGCGGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTTGGTTCTCAGTACGTGTACTGGTACCAGCAGCTGCG GGCACGGCGCCGAAACTGCTGATCTACTACAACCTCTGAACG CCGAGCGGCATGCCGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGACTTGGGACGCTTCTTCT CAGTCTTCTGTGTTTGGCGGGCGGCACGAAGTTAACCGTCTTA GGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCTGCC TCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTG

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		TCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCT GGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC CACCACACCTCCAAACAAAGCAACAACAAGTACGCGGCCA GCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCCAC AGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGT GGAGAAGACAGTGGCCCCCTACAGAATGTTCA
NOV0672	LCDR1 (Kabat) (SEQ ID NO:28)	TGTSSDVGAYNYVS
	LCDR2 (Kabat) (SEQ ID NO:29)	GVSKRPS
	LCDR3 (Kabat) (SEQ ID NO:30)	QSYDHLLHV
	HCDR1 (SEQ ID NO:31)	TVGHE
	HCDR2 (Kabat) (SEQ ID NO:32)	YIHYSGSSTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:33)	HAYGYMDF
	vH full sequence (SEQ ID NO:34)	QVQLLESGGGLVQPGGSLRLSCAASGFTFNTYGIHWVRQAPG KGLEWVSYIHYSGSSTYYADSVKGRFTISRDNSKNTLYLQMNS LRAEDTAVYYCARHAYGYMDFWGQGLVTVSS
	vL full sequence (SEQ ID NO:35)	DIALTPASVSGSPGQSITISCTGTSSDVGAYNYVSWYQQHPGK APKLMYGVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCQSYDHLLHVVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:36)	CAGGTGCAATTGCTGQAAAAGCGCGGTGGCTGGTGCAAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTAACTTACGGTATCCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCTACATCCATTACTCTGGTT CTTCTACCTACTATGCGGATAGCGTGAAAGGCCGCTTACCA TCAGCCGCGATAATTCGAAAAACACCTGTATCTGCAATG AACAGCCTGCGTGCAGGAAGATACGGCCGTGTATTATTGCGC GCGTCATGCTTACGGTTACATGGATTCTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:37)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG ATGTGGGCGCTTACAACCTACGTGTCTTGGTACCAGCAGCAT CCGGGCAAGGCGCCGAAACTGATGATCTACGGTGTCTTCTAA ACGTCCGAGCGGCGTGAGCAACCGTFTTACGGGATCCAAAA GCGGCAACACCGCGAGCCTGACCATTAGCGGCTGCAAGCG GAAGACGAAGCGGATTATTACTGCCAGTCTTACGACCATCT

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		GCTGCATGTTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT A
	HC full sequence (SEQ ID NO: 38)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFNTYGIHWVRQAPG KCLEWVSYIHYSQSSTYYADSVKGRFTISRDNSEKNTLYLQMN LRAEDTAVYYCARHAYGYMDFWQGTLLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHITCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLIHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 39)	DIALTPASVSGSPGQSITISCTGTSSDVGAYNYVSWYQQHPGK APKLMYGVSKRPSGVSNRFSQSKSGNTASLTISGLQAEDEADY YCQSYDHLHVVFTGGGKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPPSKQSN KYAASSYLSTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 40)	CAGGTGCAATTGCTGAAAAGCGGCGGTGGCTGGTGCAGCC GGGTGGCAGCCTGCGCTCTGAGCTGCGCGCGCTCCGATTCA CCTTTAACACTTACGGTATCCATTGGGTGCGCCAGGCCCGG GCAAGGTCTCGAGTGGGTTTCTACATCCATTACTCTGGT CTTCTACTACTATGCGGATAGCGTGAAAGGCCGCTTTAACA TCAGCCGCGATAATTGAAAAACACCTGTATCTGCAATG AACAGCCTGCGTGCGGAAGATACGCCCGTGATTATTGCGC GCGTCATGCTTACGGTTACATGGATTTCTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCATCG GTCTTCCCCCTGGCACCTCCTCCAAGAGCACCTTGGGGCG ACAGCGGCCCTGGGCTGCTGCTCAAGGACTACTTCCCGA ACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCG GCGTGACACACTTCCCGGCTGTCTACAGTCTCAGGACTCT ACTCCCTCAGCAGCGTGCTGACCGTGCCCTCCAGCAGCTTG GGCAGCCAGACCTACATCTGCAACCTGAATCACAAGCCAG CAACACCAAGGTGGACAAGAGAGTTGAGCCCAATCTTGTG ACAAAACCTCACACATGCCCACCGTGCCAGCACCTGAACTC CTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAA GGACACCTTCATGATCTCCCGGACCTCTGAGGTACATGCG TGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTC AACTGGTACGTGGACGCGGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTG GTCAGCGTCTCACCCTCTGACCAGGACTGGCTGAATGG CAAGGAGTACAAGTGCAAGGTCTCCAAACAAAGCCCTCCAG CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGAGGA GATGACCAAGAACCAGGTACGCTGACCTGCTGGTGAAG GCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACTACAAGACCACGCTCCCGTGCT GGACTCCGACCGCTCCTTCTTCTCTACAGCAAGCTCACCGT GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence	GATATCGCGCTGACCCAGCCGCGGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATAGCTGCACCGGCACAGCAGCG

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	(SEQ ID NO:41)	ATGTGGGCGCTTACAACTACGTGTCTTGGTACCAGCAGCAT CCGGGCAAGGCGCCGAAACTGATGATCTACGGTGTTCCTAA ACGTCCGAGCGGCGGTGAGCAACCGTTTACGGGATCCAAAA GCGGCAACACCGCGAGCCTGACCATTAGCGGCTGCAAGCG GAAGACGAAGCGGATTATTACTGCCAGTCTTACGACCATCT GCTGCATGTGTGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT AGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCTGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCTCCAAACAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCCA CAGAAGCTACAGCTGCCAGGTACGCTATGAAAGGAGCACC GTGGAGAAGACAGTGGCCCTACAGAATGTTCA
NOV0674	LCDR1 (Kabat) (SEQ ID NO:42)	SGSSSNIGYNYVS
	LCDR2 (Kabat) (SEQ ID NO:43)	RDNQRPS
	LCDR3 (SEQ ID NO:44)	AAWTSGSIGWV
	HCDR1 (Kabat) (SEQ ID NO:45)	SYAMT
	HCDR2 (Kabat) (SEQ ID NO:46)	GISGGGNTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:47)	GGGQYFDY
	vH full sequence (SEQ ID NO:48)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMFWVRQAPG KGLEWVSGISGGGNTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVVYCARGGGQYFDYWGQGT LTVSS
	vL full sequence (SEQ ID NO:49)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGYNYVSWYQQLPGTA PKLLIYRDNQRPSGVPDRFSGSKSGTSASLAFTGLQAEDADVY CAAWTSGSIGWVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:50)	CAGGTGCAATTGCTGCAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGATTCA CCTTTTCTTCTTACGCTATGACTTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTTCCGGTATCTCTGGTGGTGGTT CTAACACCTACTATGCGGATAAGCGTGAAAGGCCCGCTTACC ATCAGCCGCGATAATTGAAAAACACCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGATTATTGCG CGCGTGGTGGTGGTCACTACTTCGATTACTGGGGCCAAGGC ACCTTGGTGACTGTTAGCTCA

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	vL DNA sequence (SEQ ID NO:51)	GATATCGTGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGTGACCATTAAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTACAACCTACGTGTCTTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACCGTGACAACCAGCG CCCGAGCGGCGTGGCCGATCGCTTTAGCGGATCCAAAAGCG GCACAGCGCCAGCCTGGCGATTACCGGCTGCAAGCAGAA GACGAAGCGGATTATTACTGCGCTGCTTGGACTTCTGGTCT ATCGGTGGGTGTTTGGCGGCGGCACGAAGTTAACCCTCCT A
	HC full sequence (SEQ ID NO:52)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYAMTWVRQAPG KGLEWVSGISGGGNTYYADSVKGRFTISRDNSTLYLQMS LRAEDTAVYYCARGGGQYFDYWGQGLTVVSSASTKQPSVFP LAPSSKSTSGGTAAAGCLVYDYFEPVTVSWNSGALTSQVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHNKPSNTKVDKR VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:53)	DIVLTQPPSVSQAPGQRVTISCSGSSSNIQYNYVSWYQQLPGTA PKLLIYRDNRPSGVPDRFSGSKSGTSASLAITGLQAEADYY CAAWTSGLGWVFGGCTKLTVLGQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:54)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCTCGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGCGGTCCCGATTCA CCTTTCTTCTTACGCTATGACTTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTFTCCGGTATCTCTGGTGGTGGTT CTAACACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTGAAAAACACCTGTATCTGCAAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTAATTATGCG CGGTGGTGGTGGTCAGTACTTCGATTACTGGGGCCAAGGC ACCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCATC GGTCTTCCCCCTGGCACCTCTCCCAAGAGCACCTCTGGGGG CACAGCGGCCCTGGGCTGCTGGTCAAGGACTACTTCCCCG AACCAGGTGACGGTGTCTGGAACTCAGGCGCCCTGACCAGC GGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTC TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTG GGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCAG CAACACCAAGGTGGACAAGAGAGTTGAGCCCAAAATCTTGTG ACAAAACCTACACATGCCACCGTGCCCAAGCACCTGAACCTC CTGGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAA GGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCG TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCCGCGGAGGAGCAGTACAACAGCAGTACCGGGTG GTCAGCGTCTCACCCTGCTGACCAGGACTGGCTGAATGG CAAGGAGTACAAGTGCAAGGTCTCCAAACAAAGCCCTCCAG CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACCACAGGTGTACACCTTGCCCCCATCCCGGGAGGA GATGACCAAGAACCAGGTGAGCTGACCTGCTGGTCAAAAG

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		GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGGCAGCCGGAGAACTACAAGACCACGCCTCCCCTGCT GGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGT GGACAAGAGCAGGTGGCAGCAGGGGAACGCTCTTCTCATGCT CCGTGATGCAATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:55)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTACAACCTACGTGTCTTGGTACCAGCAGCTGECG GGCAGCGCGCCGAAACTGCTGATCTACCGTGACAACCAAGCG CCCGAGCGCGGTGCCGATCGCTTTAGCGGATCCAAAAGCG GCACCAGCGGECAGCCTGGCGATTACCGGCCTGCAAGCAGAA GACGAAGCGGATTATTAAGTGCCTGCTTGGACTTCTGGTCT ATCGGTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT AGGTCAGCCCAAGGCTGCCCTCTGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCGGGAGCGGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCCTCCAAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCCA CAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGGAGCACC GTGGAGAAGACAGTGGCCCTACAGAATGTTCA
NOV0682	LCDR1 (Kabat) (SEQ ID NO:56)	RASQTINSYLN
	LCDR2 (Kabat) (SEQ ID NO:57)	RASNLQS
	LCDR3 (SEQ ID NO:58)	QQGDSSWT
	HCDR1 (Kabat) (SEQ ID NO:59)	SYAIS
	HCDR2 (Kabat) (SEQ ID NO:60)	FIKSNADGYTTNYAAPVKG
	HCDR3 (Kabat) (SEQ ID NO:61)	IRYFRNWDY
	vH full sequence (SEQ ID NO:62)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYAISSWRQAPGK GLEWVGFIKSNADGYTTNYAAPVKGGRFTISRDDSKNTLYLQM NSLKTEDTAVYYCARIRYFRNWDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:63)	DIQMTQSPSSLSASVGDRTVITCRASQTINSYLNWYQQKPGKA PKLLIYRASNLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC QQGDSSWTFGQGTKVEIK
	vH DNA	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACC

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sequence (SEQ ID NO:64)	AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCTCCGGATTCA CCTTTTCTTCTTACGCTATCTCTTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTGGGCTTCATCAAATCTAACGCT GACGGTTACACTACTAACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCCGGATGATTTCGAAAAACACCTGTATCT GCAAATGAACAGCCTGAAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTATCCGTTACTTCCGTAACCTGGGATTACTGGG GCCAAGGCACCCCTGGTGACTGTTAGCTCA
vL DNA sequence (SEQ ID NO:65)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTAACCTGCAGAGCCAGCCAGA CTATTAACCTCTTACCTGAACTGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAATCTACCGTGCTTCTAACCTGCA AAGCGGCGTGCCGAGCCGCTTAGCGGCAGCGGATCCGGCA CCGATTTCACCTGACCATTAGCTCTCTGCAACCGGAAGACT TTGCGACCTATTATTGCCAGCAGGGTGACTCTTCTTGACCT TTGCCAGGGCAGCAAAGTTGAAATTTAA
HC full sequence (SEQ ID NO:66)	QVQLVESGGGLVKGPGSLRLSCAASGFTFSSYAISWVRQAPGK GLEWVGFISNADGYTTNYAAPVKGRFTISRDDSKNTLYLQM NSLKTEDTAVYYCARIRYFRNWDYWGQGLTVTVSSASTKGPS VFPLAPSSKSTSGGTAALOCCLKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHFTCPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVHLHQQDWLNGKEYCKCVSNKALPAPIEKTSKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SYMHEALHNHYTOKLSLSLSPGK
LC full sequence (SEQ ID NO:67)	DIQMTQSPSSLSASVGRVTITCRASQTINSYLNWYQQKPGKA PKLLIYRASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQGDSWYIFGQGTKEIKRTVAAPSVFIPPSDEQLKSGTASVY CLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSL SSITLTLSKADYERHKVYACEVTHQGLSSPVTKSFNRGEC
HC DNA sequence (SEQ ID NO:68)	CAGGTGCAATTGGTGGAAAGCGCGCGGTGGCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCTCCGGATTCA CCTTTTCTTCTTACGCTATCTCTTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTGGGCTTCATCAAATCTAACGCT GACGGTTACACTACTAACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCCGGATGATTTCGAAAAACACCTGTATCT GCAAATGAACAGCCTGAAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTATCCGTTACTTCCGTAACCTGGGATTACTGGG GCCAAGGCACCCCTGGTGACTGTTAGCTCAGCCTCCACCAAG GGTCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTA CTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGCGGCC TGACCAGCGGCGTGACACCTTCCCCTGGCTGTCTACAGTCCT CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTTGACAAAACCTACACATGCCACCGTGCCAGC ACCTGAACTCCTGGGGGACCGTCACTTCTCTTCTCCCCC AAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAG

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		GTCAAGTTCAACTGGTACGTGGACGGCGTGGAAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAGGTCCTCCAACAAA GCCCTCCCAGCCCCCATCGAGAAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCCTGCCCCCAT CCGGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACTACAAGACCACG CCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCCTCTCCCTGTCTCCGGGTAAG
	LC DNA sequence (SEQ ID NO: 69)	GATATCCAGATGACCCAGAGCCCGAGCAGCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGA CTATTAACCTTTACCTGAACCTGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAACTTACCGTGCTTCTAACCTGCA AAGCGGGCGTGCCGAGCCGCTTACCGGCGAGCGGATCCGGCA CCGATTTCACCCCTGACCATTAGCTCTCTGCAACCGGAAGACT TTGCGACCTATTATTGCCAGCAGGGTGACTCTTCTTGGACCT TTGGCCAGGGCACGAAAGTTGAAATTAAACGTACCGGTGGCC GCTCCAGCGTGTTCATCTTCCCCCAGCGACGAGCAGCTG AAGAGCGGCACCGCCAGCGTGGTGTGCCCTGCTGAACAACTT CTACCCCCCGGAGGCCAAGGTGCAGTGGGAAGGTGGACAAC GCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACCGAGC AGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCCTG ACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACG CCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGACC AAGAGCTTCAACCGGGGCGAGTGT
NOV0685	LCDR1 (Kabat) (SEQ ID NO: 70)	SGSSSNIGSYVVS
	LCDR2 (Kabat) (SEQ ID NO: 71)	YNTKRPS
	LCDR3 (Kabat) (SEQ ID NO: 72)	QSWDKLGKGYV
	HCDR1 (Kabat) (SEQ ID NO: 73)	GNSAAWN
	HCDR2 (Kabat) (SEQ ID NO: 74)	HYYSKWYNDYAVSVKS
	HCDR3 (Kabat) (SEQ ID NO: 75)	SSYSGGFDY
	vH full	QVQLQQSGPGLVKPSQTLSTCAISGDSVSGNSAAWNWIRQSP

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	sequence (SEQ ID NO:76)	SRGLEWLGIHYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLN SVTPEDTAVYYCARSSYSGGFDYWGQGTLLTVSS
	vL full sequence (SEQ ID NO:77)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSYVSWYQQLPGTA PKLLIYYNTRPSGVDPDRFSGSKSGTSASLAITGLQAEDEADYY CQSWDKLGKGYVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:78)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCGGTAACTCTGCTGCTTGGAACTGGATTCTGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCATCATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAAACAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTTCCTTACTCTGGTGGTTTCGATTAC TGGGGCCAAGGCACCCCTGGTGACTGTAGCTCA
	vL DNA sequence (SEQ ID NO:79)	GATATCGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTCTTACTACGTGCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACTACAACACTAAACGC CCGAGCGCGGTGCCGGATCGCTTTAGCGGATCCAAAAGCGG CACCAGCGCCAGCCTGGCGATTACCGGCCCTGCAAGCAGAA ACGAAAGCGGATTATTACTGCCAGTCTTGGGACAAACTGGGT AAAGGTTACGTGTTTGGCGCGCGCACGAAGTTAACCCTCCT A
	HC full sequence (SEQ ID NO:80)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSGNSAAWNWIRQSP SRGLEWLGIHYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLN SVTPEDTAVYYCARSSYSGGFDYWGQGTLLTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHPKSNITKVDK RVEPKSCDKTHTCPPCPAPELLGPPSVFLFPPKPKDITLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNST YRVVSVLTIVLHODWLNQKEYKCKVSNKALPAPIEKTIKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:81)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSYVSWYQQLPGTA PKLLIYYNTRPSGVDPDRFSGSKSGTSASLAITGLQAEDEADYY CQSWDKLGKGYVFGGGTKLTVLGGPKAAPSVTLPSPSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN KYAASSYLSTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:82)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCGGTAACTCTGCTGCTTGGAACTGGATTCTGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCATCATCTACTA CCGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAAACAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTTCCTTACTCTGGTGGTTTCGATTAC TGGGGCCAAGGCACCCCTGGTGACTGTAGCTCAGCCTCCAC CAAGGGTCCATCGGTCTTCCCCCTGGCACCCCTCTCCAAGAG CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG

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		<p>ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGC GCCCTGACCAGCGGCGTGCACACCTTCCCCGGCTGTCTTACA GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCCTGCC CTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGA ATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGA GCCCCAAATCTGTGTGACAAAACCTCAACATGCCACCCGTGCC CAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCC CCCCAAAACCCAAAGGACACCCCTCATGATCTCCCGGACCCCT GAGGTACATGCGTGGTGGTGGACCTGAGCCACGAAGACCC TGAGGTCAAGTTCAACTGGTACCTGGACGGCGTGGAGGTGC ATAATGCCAAGACAAAAGCCGCGGGAGGAGCAGTACAACAG CACGTACCGGGTGGTCAGCGTCTCACCCTCTGCACCAGG ACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC AAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGC CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTTGCCCC CATCCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACC TGCCCTGCTCAAAGGCTTCTATCCCAGCGACATCGCCGTGG GTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC ACGCTCTCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTAC AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA ACGCTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC ACTACACGCAGAAGAGCCCTCTCCCTGTCTCCGGTAAA GATATCGTGCTGACCCAGCCGCGGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCAATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTTACTACGTGTCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACTACAACACTAAACGC CCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCAG CACCAGCGCCAGCCTGGCGATTACCGCCCTGCAAGCAGAAG ACGAAGCGGATTATTAATGCTGCCAGTCTTGGGACAAAACCTGGGT AAAGGTTACGTGTTTGGCGGCGGCACGAAGTTAACCCTCCT AGGTCAGCCCAGGCTGCCCTCCTCGGTCACTCTGTTCCTGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGCGGGAGTGGAGA CCACCACACCCCTCCAAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCCA CAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCC GTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA</p>
	LC DNA sequence (SEQ ID NO: 83)	<p>GATATCGTGCTGACCCAGCCGCGGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCAATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTTACTACGTGTCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACTACAACACTAAACGC CCGAGCGGCGTGCCGGATCGCTTTAGCGGATCCAAAAGCAG CACCAGCGCCAGCCTGGCGATTACCGCCCTGCAAGCAGAAG ACGAAGCGGATTATTAATGCTGCCAGTCTTGGGACAAAACCTGGGT AAAGGTTACGTGTTTGGCGGCGGCACGAAGTTAACCCTCCT AGGTCAGCCCAGGCTGCCCTCCTCGGTCACTCTGTTCCTGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGCGGGAGTGGAGA CCACCACACCCCTCCAAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCCA CAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCC GTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA</p>
NOV0689	LCDR1 (Kabat) (SEQ ID NO: 84)	SGSSSNIGSNFVS
	LCDR2 (Kabat) (SEQ ID NO: 85)	DNSNRPS
	LCDR3 (Kabat) (SEQ ID NO: 86)	SSYDSFDHSWV
	HCDR1 (Kabat) (SEQ ID NO: 87)	SFAMN

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HCDR2 (Kabat) (SEQ ID NO: 88)	VISSSGSNTNYADSVKG
HCDR3 (Kabat) (SEQ ID NO: 89)	PSYFQAMDY
vH full sequence (SEQ ID NO: 90)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSFAMNWRQAPG KGLEWVSVISSSGSNTNYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVVYCARPSYFQAMDYWGQGTLVTVSS
vL full sequence (SEQ ID NO: 91)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNFVSWYQQLPGTAP KLLIYDNSNRPSGVPRFSGSKSGTSASLAITGLQAEDEADYYC SSYDSFDHSWVFGGGTKLTVL
vH DNA sequence (SEQ ID NO: 92)	CAGGTGCAATTGCTGAAAAGCGGCGGTGGCTGGTGACGCC GGGTGGCAGCCTGCGCTCTGAGCTGCGCGCGCTCCGGATTCA CC'TTTTCTTCTTCGCTATGAACTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTTCGGTTATCTCTTCTTCTGGTT CTAACACCAACTATGCGGATAGCGTGAAAAGCGCGCTTACC ATCAGCCGCGATAATTGCAAAAACACCTGTATCTGCAAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTCCGCTCTTACTTCCAGGCTATGGATTACTGGGGCCAAG GCACCTGGTGACTGTTAGCTCA
vL DNA sequence (SEQ ID NO: 93)	GATATCGTGTGACCCAGCCGCGCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCAATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTTCTAACTTCGTGTCTTGGTACCAGCAGCTGCCGG GCACGCGCGCGAAAAGTGGTGATCTACGACAACCTAAACCG CCGAGCGCGGTGCCGGATCGCTTTAGCGGATCCAAAAGCGG CACCAGCGCCAGCCTGGCGATTACCGGCGCTGCAAGCAGAAG ACGAAGCGGATTATTACTGCTCTTCTTACGACTCTTTCGACC ATTCTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCTA
HC full sequence (SEQ ID NO: 94)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSFAMNWRQAPG KGLEWVSVISSSGSNTNYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVVYCARPSYFQAMDYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHEKPSNTKVDK RVEPKSCDKTHITCPAPPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTSKAKG QPREPQVYTLPPSREEMITKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTIIPVLDSDGSFTLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPGK
LC full sequence (SEQ ID NO: 95)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGSNFVSWYQQLPGTAP KLLIYDNSNRPSGVPRFSGSKSGTSASLAITGLQAEDEADYYC SSYDSFDHSWVFGGGTKLTVLQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
HC DNA sequence (SEQ ID)	CAGGTGCAATTGCTGAAAAGCGGCGGTGGCTGGTGACGCC GGGTGGCAGCCTGCGCTCTGAGCTGCGCGCGCTCCGGATTCA CC'TTTTCTTCTTCGCTATGAACTGGGTGCGCCAGGCCCGG

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	NO:96)	GCAAAGGTCTCGAGTGGGTTTCGGTTATCTCTTCTCTGGTT CTAACACCAACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTCGAAAAACACCCGTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTCCGTCTTACTTCCAGGCTATGGATTACTGGGGCCAAG GCACCCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCA TCGGTCTTCCCCCTGGCACCCCTCCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCC CGAACCCGGTGACGGGTGTCGTGGAACTCAGGCGCCCTGACCA GCGGCGTGACACACTTCCCCTGGTGTCTTACAGTCTTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC CAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAATCTT GTGACAAAACCTACACATGCCACCCGTGCCCAGCACCTGAA CTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCC AAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATG CGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGT TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGG TGGTACGCGTCTCAACGTCTGCAACAGGACTGGCTGAAT GGCAAGGAGTACAAGTGCAAGGTCTECAAACAAAGCCCTCCC AGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC CCCGAGAACCACAGGTGTACACCCCTGCCCCCATCCCGGGAG GAGATGACCAAGAACAGGTACGCTGACCTGCCCTGGTCAA AGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCA ATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG CTGGACTCCGACCGGCTCTTCTTCTTCTACAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGA AGAGCCCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:97)	GATATCGTGCTGACCCAGCCGCCGAGCOTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCA ACATTGGTTCTAACTTCGTGTCTTGGTACCAGCAGCTGCCGG GCACGGCGCCGAAACTGCTGATCTACGACAACCTAAACCCG CCGAGCGGGCTGCGCGATCGCTTTAGCGGATCCAAAAGCGG CACCAGCGCCAGCCTGGCGATTACCGGCTGCAAGCAGAAG ACGAAGCGGATTATTACTGCTCTTCTTACGACTCTTTTCGACC ATTCTTGGGTGTTTGGGCGCGCACGAAGTTAACCGTCTTA GGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCTCCGCC TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTG TCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCT GGAAGGCAGATAGCAGCCCGTCAAGGCGGGAGTGGAGAC CACCACACCCCTCCAAACAAAGCAACAACAAGTACGCGGCCA GCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCAC AGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGT GGAGAAGACAGTGGCCCTACAGAATGTTCA
NOV0690	LCDR1 (Kabat) (SEQ ID NO:98)	SGDAIGTKFAH
	LCDR2 (Kabat) (SEQ ID NO:99)	YDHERPS

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LCDR3 (Kabat) (SEQ ID NO: 100)	YSRASSNLV
HCDR1 (Kabat) (SEQ ID NO: 101)	DHAID
HCDR2 (Kabat) (SEQ ID NO: 102)	VIAGDGSITYYADSVKG
HCDR3 (Kabat) (SEQ ID NO: 103)	DTGVVREYMDV
vH full sequence (SEQ ID NO: 104)	QVQLLESQGGGLVQPGGSLRLSCAASGFTTFSDHAIDWVRQAPGK GLEWVSVIAGDGSITYYADSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVVYCARDTGVVREYMDVWGQGLVTVSS
vL full sequence (SEQ ID NO: 105)	DIELTQPPSVSVSPGQTASITCSGDAIGTKFAHWYQOKPGQAPV LVIIYDHERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCY SRASSNLVPGGGTKLTVL
vH DNA sequence (SEQ ID NO: 106)	CAGGTGCAATTGCTGGAAAAGCGGCGGTGGCCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCCGGCGCTCCGGATTCA CCTTTCTGACCATGCTATCGACTGGGTGCCCCAGGCCCCCGG GCAAAGGTCTCGAGTGGGTTTCGGTATCGCTGGTGACGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTGCAAAAACACCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATAACGGCCGTGTATTATTCG CGGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCCTGGTGACTGTTAGCTCA
vL DNA sequence (SEQ ID NO: 107)	GATATCGAACTGACCCAGCCGCGGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCAATTACCTGTAGCGGCGATGCTATCG GTACTAAATTCGCTCATTGGTACCAGCAGAAACCGGCCAG GCGCCGGTGCTGGTGATCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCTACTCTCGTGCTTCTTCTAACCTGGTG TTTGGCGGCGGCACGAAGTTAACCGTCCTA
HC full sequence (SEQ ID NO: 108)	QVQLLESQGGGLVQPGGSLRLSCAASGFTTFSDHAIDWVRQAPGK GLEWVSVIAGDGSITYYADSVKGRFTISRDN SKNTLYLQMNSL RAEDTAVVYCARDTGVVREYMDVWGQGLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHITCPPCPAPPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTPFPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK

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LC full sequence (SEQ ID NO: 109)		DIELTQPPSVSVSPGQTASITCSGDAIGTKFAHWYQOKPQQAPV LVIYYDHERPFGIPERFSGSNSGNTATLTISGTQAEDEADYYCY SRASSNLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATL VCLISDFYPGAVTVAWKADSSPVKAGVETITPSKQSNKYYAA SSYLSLTPEQWKSHRSYSCOVTHIEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 110)	CAGGTGCAATTGCTGCAAAGCGGCGGTGGCCTGGTGACGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTGACCATGCTATCGACTGGGTGCGCCAGGCCCGG GCAAAGGTCCTGAGTGGGTTCCTTATCGCTGGTGACGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCCGGATAATTGCAAAAACACCCCTGTATCTGCAAA GAACAGCCTGCGTGCGGAAGATAEGGCCGTGTATTATTGCG CGCGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCCTGGTGAAGTGTAGCTCAGCCTCCACCAA GGGTCCATCGGTCTTCCCTTGGCACCCCTCCCAAGAGCAC CTCTGGGGGACACAGCGGCCCTGGGCTGGCTGGTCAAGGACT ACTTCCCGGAACCGGTGACGGTGTGCTGGAAGTCAAGCGCC CTGACCAAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCC TCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACCTACACATGCCACCGTGGCCAGC ACCTGAAGTCTCTGGGGGGACCGTCAAGTTCCTCTTCCCTCC AAAACCCAAGGACACCTCATGATCTCCCGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCTATAA TGCCAAGACAAAGCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTACCGTCTCACCCTCTGACCAAGGACTG GCTGAATGGCAAGGAGTACAAGTCAAGGTCTCCAACAAA GCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCCCTGCCCCAT CCCGGGAGGAGATGACCAAGAACCAGGTGAGCTGACCTGG CTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAAGCAACTACAAGACCAAG CCTTCCGTGCTGGACTCCGACGGCTCTCTCTCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
LC DNA sequence (SEQ ID NO: 111)		GATATCGAACTGACCCAGCCGCCGAGCGTGAACGTTGAGCC GGGCCAGACCGCGAGCATTACCTGTAGCGCGCATGCTATCG GTACTAAATTCGCTCATTGGTACCAGCAGAAACCGGCCAG GCGCCGGTGTCTGGTGATCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTACCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGCGGGAAGACGAA GCGGATTATTACTGCTACTCTCGTGTCTTCTTAACCTGGTG TTTGGCGCGCGCACGAAGTTAACCGTCTAGGTCAAGCCAA GGCTGCCCTCTCGGTACTCTGTTCCTCGCCCTCTCTGAGGA GCTTCAAGCCAAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAGGCAGAT AGCAGCCCGTCAAGGCGGGAGTGGAGACCACCACAACCTC CAAAACAAGCAACAACAAGTACCGCGCCAGCAGCTATCTG AGCCTGACCGCTGAGCACTGGAACTCCCAAGAGCTACAG CTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCCCTACAGAATGTCA

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NOV0691	LCDR1 (Kabat) (SEQ ID NO: 112)	TGTSSDVGRYNFVS
	LCDR2 (Kabat) (SEQ ID NO: 113)	RVSNRPS
	LCDR3 (Kabat) (SEQ ID NO: 114)	QSWTTYSNVV
	HCDR1 (Kabat) (SEQ ID NO: 115)	SYALN
	HCDR2 (Kabat) (SEQ ID NO: 116)	RIKSKTYGGSTDYAAPVKG
	HCDR3 (Kabat) (SEQ ID NO: 117)	DRGGYVGFD
	vH full sequence (SEQ ID NO: 118)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYALNWVRQAPG KGLEWVGRISKTYGGSTDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARDRGGYVGFDWSWGQGITLVTVSS
	vL full sequence (SEQ ID NO: 119)	DIALTPASVSGSPGQSITISCTGTSSDVGRYNFVSWYQQHPGK APKLMIRVSNRPSGVSNRFSQSKSGNTASLTISGLQAEDEADY YCQSWTTYSNVVFQGGTKLTVL
	vH DNA sequence (SEQ ID NO: 120)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCCTCCGGATTCA CCTTTTCTTCTTACGCTCTGAACCTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTGGGCGGTATCAAATCTAAAACT TACGGTGGTCTACTGACTATGCCGCCCCAGTGAAAGGCCG CTTACCATTAGCCCGGATGATTCGAAAAACACCCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTGACCGTGGTGTTACGTTGGTTTCGATTCTT GGGGCCAAGGCACCCGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO: 121)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGCACCAGCAGCG ATGTGGGCGGTACAACTTCGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAAACTGATGATCTACCGTGTTTCTAAC CGTCCGAGCGCGGTGAGCAACCGTTTTAGCGGATCCAAAAAG CGGCAACACCGCGAGCCTGACCTTAGCGGCCTGCAAGCGG AAGACGAAGCGGATTATTACTGCCAGTCTTGGACTACTTAC TCTAACCTGTGTGTTGGCGGCGGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYALNWVRQAPG KGLEWVGRISKTYGGSTDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVYYCARDRGGYVGFDWSWGQGITLVTVSSASTK

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	NO:122)	GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVPEKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRITPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQOPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVPS CSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:123)	DIALTPASVSGSPGQSITISCTGTSSDVGRYNFVSWYQHPGK APKLMYRVSNRPSGVSNRPSGSKSGNTASLTISGLQAEDEADY YCQSWTTYSNVVFQGGTKLTVLQPKAAPSVTLFPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSN KYAASSYLSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:124)	CAGGTGCAATTGGTGGAAAGCGCGCGGTGGCCCTGGTGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCCCGCCCTCCGGATTCA CCFTTCTTCTTACGCTCTGAACCTGGGTGCGCCAGGCCCCCG GCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAACT TACCGTGGTCTACTGACTATGCCGCCCCAGTGAAAGGCCG CTTACCATTAGCCGCGATGATTGAAAAACACCTTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCCGTGTATT ATTGCGCGCGTGACCGTGGTGGTTACGTTGGTTTCGATTCTT GGGGCCAAGGCACCTGGTGACTGTTAGCTCAGCCTCCACC AAGGTCCTCATCGTCTTCCCCCTGGCACCCTCCTCCAAGAGC ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGA CTACTTCCCCGAACCGGTGACCGTGTCTGTGGAACCTCAGGCG CCCTGACCAGCGCGTGACACACCTTCCCGGCTGTCTACAGT CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCT CCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT CACAAGCCCAAGCAACACCAAGGTGGACAAGAGAGTTGAGC CCAAATCTTGTGACAAAACCTACACATGCCACCGTGCCCA GCACCTGAACCTCTGGGGGACCGTCACTCTTCTCTTCCCC CCAAAACCCAAGGACACCTCATGATCTCCCGGACCCCTGA GGTCACATGCGTGGTGGTGACCTGAGCCACGAAGACCCCTG AGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA CGTACCGGGTGGTCAAGCTCTCACCCTCTGCAACAGGAC TGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAA AGCCCTCCCAAGCCCCCATCGAGAAAACCATCTCCAAGCCA AAGGGCAGCCCCGAGAACACAGGTGTACACCTTGCCCCCA TCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTG CCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGT GGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCCAC GCCTCCCGTGTCTGGACTCCGACGGCTCCTTCTTCTCTACAG CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAC GTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCCAC TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:125)	GATATCGCGCTGACCCAGCCCGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAGCTGCACCGGCACCAGCAGCG ATGTGGGCCGTTACAACTTCGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAAACTGATGATCTACCGTGTCTTAAC CGTCCGAGCGGCGTGAGCAACCGTTTTAGCGGATCCAAGAG CGGCAACACCGCGAGCCTGACCAATTAGCGGCTTGAAGCGG AAGACGAAGCGGATTATTACTGCCAGTCTTGGACTACTTAC TCTAACGTTGTGTTTGGCGCGGCACGAAGTTAACCGTCTTA

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		GGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCTCCGCCC TCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTG TCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCT GGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC CACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCA GCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCAC AGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGT GGAGAAGACAGTGGCCCCCTACAGAATGTTCA
NOV0692	LCDR1 (Kabat) (SEQ ID NO: 126)	SGDSIGSKY AQ
	LCDR2 (Kabat) (SEQ ID NO: 127)	YNSERPS
	LCDR3 (Kabat) (SEQ ID NO: 128)	QSWDGQSTIRV
	HCDR1 (Kabat) (SEQ ID NO: 129)	RYWMD
	HCDR2 (Kabat) (SEQ ID NO: 130)	RIKSKANGGITDYAAPVKG
	HCDR3 (Kabat) (SEQ ID NO: 131)	GMTFLGI
	vH full sequence (SEQ ID NO: 132)	QVQLVESGGGLVKPGGSLRLSCAASGFTFSRYWMDWVRQAP GKGLEWVGRISKANGGITDYAAPVKGRTISRDDSKNTLYLQ MNSLKTEDTAVYYCARGMTFLGIWQQGTLVTVSS
	vL full sequence (SEQ ID NO: 133)	DIELTQPPSVSVSPGQTASITCSGDSIGSKY AQWYQQKPGQAPV LVIIYNSEPSGIPERPSGNSGNTATLTISGTQAEDEADYYCQS WDGQSTIRVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO: 134)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCTCCGGATTCA CCTTTTCTCGTTACTGGATGGACTGGGTGCGCCAGGCCCGG GCAAAGGTCTGAGTGGGTGGGCCGTATCAAATCTAAAGCT AACGGTGGTATCACTGACTATGCCGCCCCAGTGAAAGGCCG CTTACCATTAGCCGCGATGATTCGAAAAACACCCCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCGGTGTATT ATTGCGCGCGTGGTATGACTTTCTGGGTATCTGGGGCCAA GGCACCCCTGGTGA CTGTTAGCTCA
	vL DNA sequence (SEQ ID)	GATAICGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCA TTACCTGTAGCGCGGATTCTATCG GTTCTAAATACGCTCAGTGGTACCAGCAGAAACCGGGCCAG

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	NO:135)	GCGCCGGTGCTGGTGATCTACTACAACTCTGAACGTCCTGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCTGTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGGTCAGTCTACTATC CGTGTGTTTGGCGCGGACCGAAGTTAACCCTGCTA
	HC full sequence (SEQ ID NO:136)	QVQLVESGGGLVLPKPGGSLRLSCAASGFTFSRYWMDWVRQAP GKGLEWVGRIKSKANGGITDYAAPVKGRFTISRDDSKNTLYLQ MNSLKTEDTAVVYCARGMIFLGIWGGGTLVTVSSASTGQPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TPPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KRVEPKSCDKTHITCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLEHQQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMITKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVPSCS VMHEALHNHYTQKSLSPGK
	LC full sequence (SEQ ID NO:137)	DIELTQPPSVSVSPGQTASITCSGDSIGSKYAQWYQOKPGQAPV LVIYYNSERPSGIPERPSGNSNGNTATLITSGTQAEDEADYYCQS WDGQSTIRVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPYKAGVETITPSKQSNKNKYA ASSYLSLTPEQWKSHRSYSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:138)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCTGGTGGAAACC AGGCGGCAGCCTGCGCCTGAGCTGCGCCGCTCCGGATTCA CCTTTTCTCGTTACTGGATGGACTGGGTGCGCCAGGCCCCCG GCAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAGCT AACGGTGGTATCACTGACTATGCCGCCCCAGTGAAAGGCCG CTTTACCATTAGCCCGCATGATTCGAAAAACACCTGTATCT GCAAATGAACAGCCTGAAAACCGAAGATACGGCGGTGATT ATTGCGCGCGTGTATGACTTTCTGGGTATCTGGGGCCAA GGCACCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCC ATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACCTCTGG GGGCACAGCGGCCCTGGGCTGCCCTGGTCAAGGACTACTTCC CCGAACCGGTGACGGTGTCTGTGAACTCAGGCGCCTGACC AGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGG ACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCA GCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG CCCAGCAACACCAAGGTGGACAAAGAGAGTTGAGGCCAAAT CTTGTGACAAAACTCACACATGCCACCGTGCCACGACCT GAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAA CCCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTAC ATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCA AGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCC AAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACC GGGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT CCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGC AGCCCCGAGAACACAGGTGTACACCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTACGCTGACCTGCTGGT CAAAGGCTTCTATCCACAGGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACAAGACCAGCCTCCC GTGCTGGACTCCGACGGCTCTTCTTCTCTACAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGC

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		AGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO: 139)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCAATTACCTGTAGCCGCGATTCTATCG GTTCTAAATACGCTCAGTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACTACAACCTCTGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAAGCGGCAACA CCGCGACCCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGGTCAGTCTACTATC CGTGTGTTTGGCGGCGGCACGAAGTTAACCCTCTAGGTCA GCCCAAGGCCTGCCCCCTCGGTCACTCTGTTCGCCCTCTCTC TGAGGAGCTTCAAGCCAACAAGGCCCACTGGTGTGTCTCA TAAGTGACTTCTACCCGGGAGCCGTGACAGTGCCCTGGAAG GCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA CACCTCCAAACAAAGCAACAACAAGTACCGCGCCAGCAG CTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCCACAGAA GCTACAGCTGCCAGGTCAACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCTACAGAATGTTCA
NOV0693	LCDR1 (Kabat) (SEQ ID NO: 140)	RASQISIFYLA
	LCDR2 (Kabat) (SEQ ID NO: 141)	GASTLQS
	LCDR3 (Kabat) (SEQ ID NO: 142)	HQYSYWLRT
	HCDR1 (Kabat) (SEQ ID NO: 143)	SYALH
	HCDR2 (Kabat) (SEQ ID NO: 144)	YIFYDSSSTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO: 145)	FLYSAYGVAN
	vH full sequence (SEQ ID NO: 146)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSYALHWVRQAPG KGLEWVSYIFYDSSSTYYADSVKGRFTISRDNKNTLYLQMNS LRAEDTAVYYCARFLYSAYGVANWGQGILVTVSS
	vL full sequence (SEQ ID NO: 147)	DIQMTQSPSSLSASVGDRTVITFCRASQISIFYLAWYQKPGKAP KLLIYGASTLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCH QYSYWLRTFGQGTKEIK
	vH DNA sequence (SEQ ID NO: 148)	CAGGTGCAATTGCTGGAAAAGCGGCGGTGGCCCTGGTGACGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTCTGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTTCTTACATCTTCTACGACTCTT CTTCTACCTACTATGCGGATAGCGTGAAAGGCCGCTTACCA

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		TCAGCCCGGATAAATTCGAAAAACACCCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTTTCTGTACTCTGCTTACGGTGTTGCTAACTGGGGCCA AGGCACCCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO: 149)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCAATFACCTGCAGAGCCAGCCAGT CTATTTCTTTCTACCTGGCTTGGTACCAGCAGAAACCGGCCA AAGCGCCGAAACTATTAATCTACGGTGCTTCTACTCTGCAA AGCGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCAC CGATTTCACCCCTGACCATTAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTAATTGCCATCAGTACTCTTACTGGCTGCGTAC CTTTGGCCAGGGCACGAAAGTTGAAATTA
	HC full sequence (SEQ ID NO: 150)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYALHWVRQAPG KGLEWVSYIFYDSSSYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVVYCARFLYSAYGVANWGQGLTVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TTPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVD KRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT P EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVQLHQQDWLNGKEYKCKVSNKALPAPIEKTIISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 151)	DIQMTQSPSSLSASVGRVTITCRASQISIFYLAWYQQKPKAP KLLIYGASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCH QYSYWLRTFGQGTKEIKRTVAAPSVEIFPPSDEQLKSGTASVV CLLNFEYPREAKVQWKVDNALQSGNSQESVTEQDSKDSFYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO: 152)	CAGGTGCAATTCCTGCAAAAGCGGCGGTGGCTTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGCTCCGGATTCA CCTTTTCTTCTTACGCTCTGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCTCGAGTGGGTTCCTACATCTTCTACGACTCTT CTTCTACTACTATGCGGATAGCGTGAAAGGCCGCTTTACCA TCAGCCCGGATAAATTCGAAAAACACCCCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTTTCTGTACTCTGCTTACGGTGTTGCTAACTGGGGCCA AGGCACCCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTC CATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTG GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC CCCGAACCCTGGTGACGGGTGCTGCGGAACTCAGCGGCCCTGAC CAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC AGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAA GCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCAAA TCTTGTGACAAAACTCACACATGCCACCGTGCCCAAGCACC TGAACCTCTGGGGGACCGTCAGTCTTCTCTTCCCCC AAA ACCCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTCA CATGCGTGCTGGTGACGTGAGCCACGAAGACCTGAGGTG AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGAGGAGCAGTACAACAGCAGTAC CGGGTGGTCAGCGTCTCACCCTCTGCAACAGGACTGGCT GAATGCCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCC

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		CTCCCAAGCCCCATCGAGAAAAACCATCTCCAAAGCCAAAGG GCAGCCCCGAGAAACCACAGGTGTACACCTGCCCCCATCCC GGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCCTG GTCAAAGGCTTCTATCCAGCGACATCGCGTGGAGTGGGA GAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACA CGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO: 153)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGT CTATTTCTTTCTACCTGGCTTGGTAAACAGCAGAAACCGGGCA AAGCGCCGAAACTATTAATCTACGGTGCTTCTACTCTGCAA AGCGGGCGTGCCGAGCCGCTTTAGCGGCAGCGGATCCGGCAC CGATTTCACCCTGACCATTAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTATTGCCATCAGTACTCTTACTGGCTGGGTAC CTTTGGCCAGGGCACGAAAGTTGAAATTAACCGTACGGTGG CCGCTCCCAAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGC TGAAGAGCGGCACCGCCAGCGTGGTGTGCTGCTGAACAAC TTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAAGGTGGACAA CGCCCTGCAGAGCGGGCAACAGCCAGGAAAGCGTCACCGAG CAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCTT GACCTGAGCAAGGCCGACTACGAGAAGCACAAAGGTGTAC GCCGTGCGAGGTGACCCACCAGGGCTTGTCCAGCCCCGTGAC CAAGAGCTTCAACCGGGGCGAGTGT
NOV0695	LCDR1 (Kabat) (SEQ ID NO: 154)	RASQGIFTYLN
	LCDR2 (Kabat) (SEQ ID NO: 155)	AASTLQS
	LCDR3 (Kabat) (SEQ ID NO: 156)	QQYYSTSLT
	HCDR1 (Kabat) (SEQ ID NO: 157)	SNSAAWN
	HCDR2 (Kabat) (SEQ ID NO: 158)	RIYYRSKWYNDYAVSVKS
	HCDR3(Ka bat) (SEQ ID NO: 159)	ERSYRDYFDY
	vH full sequence (SEQ ID NO: 160)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDYSKNQFSLQL NSVTPEDTAVYYCARERSYRDYFDYWGQGLVTVSS
	vL full	DIQMTQSPSSLSASVGDRTVITCRASQGIFTYLNWYQQKPGKA

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	sequence (SEQ ID NO: 161)	PKLLISAASTLQSGVPSRFSGSGSGTDFTLTISSLOPEDFATYYC QYYSTSLTFGQGTKVEIK
	vH DNA sequence (SEQ ID NO: 162)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCTGAGCCTGACCTGCCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAAGTGGATTCTCAGA GCCCCAGCCGTGGCTTCGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCCGCGCTGAACGTTCTTACCGTGACTACTTCGAT TACTGGGGCCAAGGCACCCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO: 163)	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCAATACCTGCAGAGCCAGCCAGG GTATTTTCACTTACCTGAACGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAATCTCTGCTGCTTCTACTCTGCAA AGCGGCGTGCCGAGCCGCTTTAGCGGCACGGGATCCGGCAC CGATTTCACCTGACCAATTAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTATTGCCAGCAGTACTACTCTACTTCTCTGAC CTTGGCCAGGGCACGAAAGTTGAAATTA
	HC full sequence (SEQ ID NO: 164)	QVQLQSGPGLVKPSQTLSTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRIYYRSK WYNDYAVSVKSRITINPDTSKNQPSLQL NSVTPEDTAVYYCARERSYRDYFDYWOQGLTVTVSSASTKGP SVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSG VHITPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHHKPSNTK VDKRVEPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 165)	DIQMTQSPSSLSASVGDVRVTHCRASQGIFTYLNWYQQKPGKA PKLLISAASTLQSGVPSRFSGSGSGTDFTLTISSLOPEDFATYYC QYYSTSLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTYS LSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO: 166)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCTGAGCCTGACCTGCCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAAGTGGATTCTCAGA GCCCCAGCCGTGGCTTCGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCCGCGCTGAACGTTCTTACCGTGACTACTTCGAT TACTGGGGCCAAGGCACCCCTGGTGACTGTTAGCTCAGCCTC CACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCCTCCTCAA GAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCTTGGTCA AGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCA GGCGCCCTGACCAGCGCGGTGCACACCTTCCCCGGCTGTCTT ACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCG TGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAG

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		TTGAGCCCCAAATCTTGTGACAAAACCTCACACATGCCACCG TGCCACAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCCTC TTCCCCCAAAAACCCAAAGGACACCCCTCATGATCTCCCGGAC CCCCTGAGGTACATGCGTGGTGGTGGAGCTGAGCCACGAAG ACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG GTGCATAATGCCAAGACAAAAGCCGCGGGAGGAGCAGTACA ACAGCACGTACCGGGTGGTCAGCGTCTCACCCTCCTGCAC CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC CAACAAAGCCCTCCAGCCCCCATCGAGAAAAACCATCTCCA AAGCCAAAGGGCAGCECCGAGAACACAGGTGTACACCT GCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCC TGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCC GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACA AGACCACGCCCTCCCGTGGTGGACTCCGACGGCTCTCTTCC TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG GGGAAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC AACCCTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAA A
	LC DNA sequence (SEQ ID NO: 167)	GATATCCAGATGACCCAGAGCCCCGAGCAGCCTGAGCGCCAG CGTGGGCGATCGCGTGACCAATACCTGCAGAGCCAGCCAGG GTATTTTCACTTACCTGAACCTGGTACCAGCAGAAACCGGGC AAAGCGCCGAAACTATTAATCTCTGCTGCTTCTACTCTGCAA AGCGGCGTGCCGAGCCGCTTTAGCGGCGAGCGGATCCGGCAE CGATTTCACCTTGACCAATAGCTCTCTGCAACCGGAAGACTT TGCGACCTATTATTGCCAGCAGTACTACTCTACTTCTCTGAC CTTTGCCCAAGGGCACGAAAGTTGAAATTAAACGTACGGTGG CCGCTCCCAAGCGTGTTCATCTTCCCCCCCCAGCGACGAGCAGC TGAAGAGCGGCACCGCCAGCGTGGTGTGCTGCTGAAACAAC TTCTACCECCGGGAGGCCAAGGTGCAAGTGGAAAGGTGGACAA CGCCCTGCAGAGCGGCCAACAGCCAGGAAAGCGTCACCGAG CAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCTCT GACCTGAGCAAGGCGGACTACGAGAAGCACAAAGGTGTAC GCCTGCGAGGTGACCCACCAGGGCCCTGTCCAGCCCCGTGAC CAAGAGCTTCAACCGGGCGAGTGT
NOV0699	LCDR1 (Kabat) (SEQ ID NO: 168)	SGDNIRKYVVH
	LCDR2 (Kabat) (SEQ ID NO: 169)	RDNNRPS
	LCDR3 (Kabat) (SEQ ID NO: 170)	QSWDSFLAVV
	HCDR1 (Kabat) (SEQ ID NO: 171)	SYAMH
	HCDR2 (Kabat) (SEQ ID NO: 172)	FISLGSYTTYADSVKG

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	HCDR3 (Kabat) (SEQ ID NO: 173)	ETAGYGYAFDP
	vH full sequence (SEQ ID NO: 174)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPG KGLEWVSFISSLGSYTYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARETAGYGYAFDPWGQGTLLVTVSS
	vL full sequence (SEQ ID NO: 175)	DIELTQPPSVSVSPGQTASITCSGDNIRKYV VHWYQQKPGQAP VLVIYRDNNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYC QSWDSFLAVVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO: 176)	CAGGTGCAATTGCTGGAAAAGCGGCGGTGGCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTATGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCCTGAGTGCGGTTCTTCTCATCTCTTCTCTGGGT CTTACACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTGGAACAAACACCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG CGCGTGAACCTGCTGGTTACGGTTACGCTTTTCGATCCGTGGG GCCAAGGCACCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO: 177)	GATATCGAACTGACCCAGCCGCGGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGCGGATAACATCC GTAAATACGTTGTTTATTGOTACCAGCAGAAACCGGCCAG GCGCCGGTGCTGGTGATCTACCGTGACAACAACCGTCCGAG CGGCATCCCGGAACGTTTATAGCGGATCCAACAGCGGCAACA CCGCGACCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACTCTTCTCTGGCTGTT TGTTTGGCGCGCGCACGAAGTTAACCGTCCTA
	HC full sequence (SEQ ID NO: 178)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPG KGLEWVSFISSLGSYTYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARETAGYGYAFDPWGQGTLLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNIHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDITLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK RGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO: 179)	DIELTQPPSVSVSPGQTASITCSGDNIRKYV VHWYQQKPGQAP VLVIYRDNNRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYC QSWDSFLAVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKY AASSYLSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 180)	CAGGTGCAATTGCTGGAAAAGCGGCGGTGGCTGGTGCAGCC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTTCTTACGCTATGCATTGGGTGCGCCAGGCCCCGG GCAAAGGTCCTGAGTGCGGTTCTTCTCATCTCTTCTCTGGGT CTTACACCTACTATGCGGATAGCGTGAAAGGCCGCTTTACC ATCAGCCGCGATAATTGGAACAAACACCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCG

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		CGCGTGAAACTGCTGGTTACGGTTACGCTTTTCGATCCGTGGG GCCAAGGCACCCCTGGTGACTGTTAGCTCAGCCTCCACCAAG GGTCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACC TCTGGGGGCACAGCGGCCCTGGGCTGCTGCTGTTCAAGGACTA CTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCC TGACCAGCGGGCTGCACACCTTCCCCGGCTGTCTTACAGTCTT CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTTGTGACAAAACCTCACACATGCCCAACCGTGCCAGC ACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCC AAAACCCAAGGACACCTTCATGATCTCCCGGACCCCTGAGG TCACATGCTGGTGGTGGACGTGAGCCACGAAGACCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCGCGGGGAGGAGCAGTACAACAGCAG TACCGGGTGGTCAGCGTCTTACCCGTCTTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCTGTCCCCCAT CCGGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACTACAAGACCAAG CTCCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAAGAGCCTCTCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO: 181)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGCGGATAACATCC GTAAATACGTTGTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGTGGTGATCTACCGTGACAACAACCGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCTTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACTCTTTCCTGGCTGTT GTGTTTGGCGGCGGCACGAAGTTAACCGTCCTAGGTACGCC CAAGGCTGCCCCCTCGGTCACTCTGTTCCCCGCCCTCCTCTGA GGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAA GTGACTTCTACCCGGAGCCGTGACAGTGGCCTGGAAGGCA GATAGCAGCCCCGTCAAGBCGGGAGTGGAGACCACACAC CCTCCAAACAAGCAACAACAAGTACCGCGCCAGCAGCTAT CTGAGCCTGACGCCCTGAGCAGTGGAAAGTCCCACAGAAGCTA CAGCTGCCAGGTACGCATGAAGGGAGCACCGTGGAGAAAG ACAGTGGCCCCCTACAGAATGTTC
NOV0705	LCDR1 (Kabat) (SEQ ID NO: 182)	SGSSSNIGLDYVN
	LCDR2 (Kabat) (SEQ ID NO: 183)	RNKQRPS
	LCDR3 (Kabat) (SEQ ID NO: 184)	QAWAGRTNYVV

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HCDR1 (Kabat) (SEQ ID NO: 185)	NYWIG
HCDR2 (Kabat) (SEQ ID NO: 186)	FIDPGVSYTRYSPSPQG
HCDR3 (Kabat) (SEQ ID NO: 187)	VLAHSTEYNWPAF
vH full sequence (SEQ ID NO: 188)	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWIGWVRQMPG KGLEWMGFIDPGVSYTRYSPSPQGGQVTISADKSISTAYLQWSSL KASDTAMYCARVLAHSTEYNWPAFWGQGTILVTVSS
vL full sequence (SEQ ID NO: 189)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGLDYVNWYQQLPGTA PKLLIYRNKQRPSPGVDRFSGSKSGTSASLAITGLQAEDEADYY CQAWAGRTNYVVFGGGTKLTVL
vH DNA sequence (SEQ ID NO: 190)	CAGGTGCAATTGGTGCAGAGCGGTGCGGAAAGTGAAAAAAC CGGGCGAAAGCCTGAAAATTAGCTGCAAAGGCTCCGGATAT AGCTTCACTAACTACTGGATCGGTGGGTGCGCCAGATGCC GGGCAAAGGTCTCGAGTGGATGGGCTTCATCGACCCGGGTG TTAGCTACACCCGTTATAGCCCGAGCTTTCAGGGCCAGGTG ACCATTAGCGCGGATAAAAGCATCAGCACCGCGTATCTGCA ATGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTATTAAT GCGCGCGTGTCTGGCTCATTCTACTGAATACAACCTGCCCGG CTTCTCGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
vL DNA sequence (SEQ ID NO: 191)	GATAICGTGCTGACCCAGCCGCCGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCA ACATTGGTCTGGACTACGTGAAGTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACCGTAACAAACAGCG CCCGAGCGCGGTGCCGGATCGCTTACCGGATCCAAAAGCG GCACCAAGCGCCAGCCTGGCGATTACCGGCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGGCTTGGGCTGGTCTGTA TAACTACGTTGTGTTGGCGCGGCACGAAGTTAACCGTCTT A
HC full sequence (SEQ ID NO: 192)	QVQLVQSGAEVKKPGESLKISCKGSGYSFTNYWIGWVRQMPG KGLEWMGFIDPGVSYTRYSPSPQGGQVTISADKSISTAYLQWSSL KASDTAMYCARVLAHSTEYNWPAFWGQGTILVTVSSASTKG PSVFPPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VRIHPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTK VDRKVEPKSCDKTHITCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDYSHEDPEVKFNWYVDQVEVHNAKTKPREEQ YNSTYRVVSVLTVLIHQDWLNGKEYKCKVSNKALPAPIEKTSK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
LC full sequence (SEQ ID NO: 193)	DIVLTQPPSVSGAPGQRVTISCSGSSSNIGLDYVNWYQQLPGTA PKLLIYRNKQRPSPGVDRFSGSKSGTSASLAITGLQAEDEADYY CQAWAGRTNYVVFGGGTKLTVLGGPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSN

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		NKYAASSYL.SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO: 194)	CAGGTGCAATTTGGTGCAGAGCGGTGCGGAAAGTGAAAAAAC CGGGCGAAAGCCTGAAAATTAGCTGCAAAGGCTCCGGATAT AGCTTCACTAACTACTGGATCGGTTGGGTGCGCCAGATGCC GGGCAAAGGTCTCGAGTGGATGGGCTTCATCGACCCGGGTG TTAGCTACACCCGTTATAGCCCGAGCTTTCAGGGCCAGGTG ACCATFAGCGCGGATAAAAAGCATCAGCACCCGGTATCTGCA ATGGAGCAGCCTGAAAGCGAGCGATACCGCGATGTATTATT GCGCGCGTGTCTGGCTCATTCTACTGAATACAACCTGCCCGG CTTCTTGGGGCCAAAGGCACCCCTGGTGAAGTGTAGCTCAGCCT CCACCAAGGGTCCATCGGTCTTCCCCCTGGCACCCCTCCCTCA AGAGCACCTCTGGGGGACAGCGGCCCTGGGCTGCTTGGTC AAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAATC AGGCGCCCTGACCAGCGCGGTGCACACCTTCCCCGGCTGTCC TACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACC GTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA CCTGAATCACAAGCCCAAGCAACACCAAGGTGGACAAGAGA GTTGAGGCCAAATCTTGTGACAAAACTCACACATGCCCAAC GTGCCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCC TCTTCCCCCAAAACCCAAGGACACCCCTCATGATCTCCCCG ACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGA AGACCTTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA CAACAGCACGTACCGGGTGGTCAGCGTCTCACCCTCCTGTC ACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGT CTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAAACCATCT CCAAAGCCAAAGGGCAGCCCTGAGAACACAGGTGTACAC CCTGCCCCCATCCCGGGAGGAGATGACCAAGAACACAGGTCA GCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGACAACCT ACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCT TCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAAGAGCCTCTCCCTGTCTCCGG GTAAA
	LC DNA sequence (SEQ ID NO: 195)	GATATCGTGCTGACCCAGCCGCGGAGCGTGAGCGGTGCACC GGGCCAGCGCGTGACCATTAGCTGTAGCGGCAGCAGCAGCA ACATTGGTCTGGACTACGTGAACCTGGTACCAGCAGCTGCCG GGCACGGCGCCGAAACTGCTGATCTACCGTAACAAACAGCG CCCGAGCGGCGTGCCGGATCGCTTACCGGATCCAAAAGCG GCACCAGCGCCAGCCTGGCGATTACCGGCCCTGCAAGCAGAA GACGAAGCGGATTATTACTGCCAGGCTTGGGCTGGTCTGTAC TAACTACGTTGTGTTTGGCGCGCGCACGAAGTTAACCGTCT AGGTACAGCCCAAGGCTGCCCTCCGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAAACAAGGCCACACTGGTGT GTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCCTCCAAACAAAGCAACAACAAGTACCGGGCC AGCAGCTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCCA CAGAAGCTACAGCTGCCAGGTACCGCATGAAAGGAGCACC GTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA
NOV0709	LCDR1 (Kabat) (SEQ ID	TGTSSDVGSYNYVS

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	NO:196)	
	LCDR2 (Kabat) (SEQ ID NO:197)	YVSNRPS
	LCDR3 (Kabat) (SEQ ID NO:198)	ASYTHQGSWV
	HCDR1 (Kabat) (SEQ ID NO:199)	TYVMH
	HCDR2 (Kabat) (SEQ ID NO:200)	VISSDGSFTFYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:201)	HGYGAFDY
	vH full sequence (SEQ ID NO:202)	QVQLLES GGGLVQPGGSLRLSCAASGFTFSTYYMHWVRQAPG KGLEWVSVISSDGSFTFYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARHGYGAFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:203)	DIALTPASVSGSPGQSITISCTGTSSDVGSYNYVSWYQQHPGK APKLMIIYYVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCASYTHQGSWVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:204)	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCCTGGTGCAGCC GGGTGGCAGCCTGCCCTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTACTTACTACATGCATTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCTCTTCTGACGGTT CTTTCACCTTCTATGCGGATAGCGTGAAAGGCCCGCTTACCA TCAGCCCGGATAAATTCGAAAAACACCTGTATCTGCAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTCATGGTTACGGTGCTTTTCGATTACTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCA
	vL DNA sequence (SEQ ID NO:205)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCAATTACCATTAGCTGCACCGGCACCAAGCAGCG ATGTGGGCTCTTACAACTACGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAAACTGATGATCTACTACGTTTCTAAC CGTCCGAGCGCGGTGAGCAACCGTTTTAGCGGATCCAAAAG CGGCAACACCGCGAGCCTGACCATTAGCGGCCTGCAAGCGG AAGACGAAGCGGATTATTACTGCGCTTCTTACACTCATCAG GGTTCTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCCT A
	HC full sequence (SEQ ID NO:206)	QVQLLES GGGLVQPGGSLRLSCAASGFTFSTYYMHWVRQAPG KGLEWVSVISSDGSFTFYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARHGYGAFDYWGQGTLVTVSSASTKGPSVF LAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKR

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		VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKISKAKGQ PREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:207)	DIALTPASVSCSPGQSITHSCTGTSSDVGSYNYVSWYQOHPGK APKLMIIYYVSNRPSGVSNRFSQSKSGNTASLTISGLQAEDEADY YCASYTHQGSWVFGGKLTVLGQPKAAPSVTLFPPPSSEELQA NKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN KYAASSYLSLTPEQWKSHRSYSQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:208)	CAGGTGCAATTGCTGGAAAAGCGCGGTGGCCTGGTGCAAGC GGGTGGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTACTTACTACATGCAATTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTTTCCGTTATCTCTTCTGACGGTT CTTTCACCTTCTATGCGGATAGCGTGAAAGGCCCGCTTACCA TCAGCCGCGATAATTGAAAAACACCTGTATCTGCAAATG AACAGCCTGCGTGCGGAAGATACGGCCGTGTATTATTGCGC GCGTCATGGTTACGGTGTCTTTCGATTACTGGGGCCAAGGCA CCCTGGTGACTGTTAGCTCAGCCTCCACCAAGGGTCCATCG GTCTTCCCCCTGGCACCTCTCTCCAAAGAGCACCTCTGGGGGC ACAGCGGCCCTGGGCTGCTTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTGCTGGAATCAGGCGCCCTGACCAAGCG GCGTGACACCTTCCCGCTGTCTTACAGTCTCAGGACTCT ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTG GGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG ACAAAACTCACACATGCCACCGTGCCCAAGCAGCTGAACTC CTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAA GGACACCTTCATGATCTCCCGGACCCCTGAGGTCAATGCG TGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGAC AAAGCGCGGGAGGAGCAGTACAACAGCAGCTACCGGGTG GTCAGCGTCTCACCGTCTTGCAACAGGACTGGCTGAATGG CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC CGAGAACACAGGTGTACACCTTGCCCCCATCCCGGGAGGA GATGACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAAG GCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAAT GGCAGCGGAGAACTACAAGACCAAGCTCCCGTGGT GGACTCCGACGGCTCTTCTTCTCTACAGCAAGCTCACCGT GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:209)	GATATCGCGCTGACCCAGCCGGCGAGCGTGAGCGGTAGCCC GGGCCAGAGCATTACCATTAAGCTGCAACCGCACCAAGCAG ATGTGGGTCTTACAACCTACGTGTCTTGGTACCAGCAGCATC CGGGCAAGGCGCCGAACTGATGATCTACTACGTTTCTAAC CGTCCGAGCGGCGTGAGCAACCGTTTACGCGATCCAAAAG CGGCAACACCGCGAGCTGACCAATTAGCGGCTGCAAAGCG AAGACGAAGCGGATTATTACTGCGCTTCTTACACTCATCAG GGTCTTGGGTGTTTGGCGGCGGCACGAAGTTAACCGTCT AGGTACAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCC CTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGT

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		GTCTCATAAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCC TGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGA CCACCACACCCCTCCAAACAAAGCAACAACAAGTACGCGGCC AGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAAGTCCCA CAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACC GTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA
NOV0710	LCDR1 (Kabat) (SEQ ID NO:210)	RASQSISLWLN
	LCDR2 (Kabat) (SEQ ID NO:211)	AASTLQS
	LCDR3 (Kabat) (SEQ ID NO:212)	QYYTSPYT
	HCDR1 (Kabat) (SEQ ID NO:213)	SYAMS
	HCDR2 (Kabat) (SEQ ID NO:214)	VIRSSGSSTYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:215)	GGGYFDY
	vH full sequence (SEQ ID NO:216)	QVQLLESQGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSVIRSSGSSTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARGGGYFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:217)	DIQMTQSPSSLSASVGDRTVITCRASQSISLWLNWYQKPKGA PKLLIYAASLTQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC QYYTSPYTFGQGTKVEIK
	vH DNA sequence (SEQ ID NO:218)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAACC TGGCGGATCCCTGAGGCTGAGCTGCGCTGCTAGTGGCTTCA CCTTCTCTAGCTACGCTATGAGCTGGGTCCGCCAGGCCCTG GTAAAGGCCTCGAGTGGGTGTCACTGATTAGATCTAGCGGC TCTAGCACCTACTACGCCGATAGCGTGAAGGCCCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCCTGTACCTGCAGA TGAACTCCCTGAGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGAGGCGGAGGCTACTTCGACTACTGGGGTCAAGGCAC CCTGGTCACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:219)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATTAGCCTGTGGCTGAAGTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGCTCTAECCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCTGACTATCTCTAGCCTGCAGCCCGAGGACT

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		TCGCTACCTACTACTGTCAGCAGTACTACACTAGCCCCCTACA CCTTCGGTCAGGGCACTAAGGTCGAGATTAAG
	HC full sequence (SEQ ID NO:220)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KCLEWVSVIRSSGSTYYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARGGGYFDYWGQGLVTVSSASTKGPSVFL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKR VEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDITLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSIDGSFFLYSKLTVDKSRWQQGNVFCFSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:221)	DIQMTQSPSSLSASVGRVTITCRASQISLWLNWYQKPGKA PKLLIYAASITLQSGVPSRFSQSGSGTDFTLTISSLQPEDPATYYC QQYYTSPYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:222)	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGC AACC TGGCGGATCCCTGAGGCTGAGCTGCGCTGCTAGTGCTTCA CCTTCTCTAGCTACGCTATGAGCTGGGTCCGCCAGGCCCTG GTAAAGGCCTCGAGTGGGTGTCACTGATTAGATCTACGCGC TCTAGCACCTACTACGCCGATAGCGTGAAGGCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCCTGTACCTGCAGA TGAACCTCCCTGAGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGAGGCGGAGGCTACTTCGACTACTGGGGTCAAGGCAC CCTGGTCACCGTGTCTAGCGCTAGC ACTAAGGGCCCAAGTG TGTTTCCCTTGCCCCCAGCAGCAAGTCTACTTCCGCGCGAA CTGCTGCCCTGGGTGCTTGGTGAAGGACTACTTCCCCGAGC CCGTGACAGTGTCTTGAACCTCTGGGGCTCTGACTTCCGGC GTGCACACCTTCCCCCGCGTGTGTCAGAGCAGCGGCTGTGA CAGCCTGAGCAGCGTGGTGACAGTGCCTCCAGCTCTCTGG GAACCCAGACCTATATCTGCAACGTGAACCAAGCCCAAGC AACACCAAGGTGGACAAGAGAGTGGAGCCCAAGGCTCGC ACAAGACCCACACCTGCCCCCCTGCCAGCTCCAGAACTG CTGGGAGGGCCCTTCCGTGTTCTGTTCCTCCCAAGCCCAAG GACACCTGATGATCAGCAGGACCCCGAGGTGACCTGCGT GGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGAAGTTCA ACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACC AAGCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGG TGTCCGTGCTGACCGTGTGTCACAGGACTGGCTGAACGGC AAAGAATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCAGC CCCAATCGAAAAGACAATCAGCAAGGCCAAGGGCCAGCCA CGGGAGCCCCAGGTGTACACCTGCCCCCAGCCGGGAGGA GATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGG GCTTCTACCCAGCGATATCGCCGTGGAAGTGGGAGCAAC GGCCAGCCCCGAGAACA ACTACAAGACCACCCCCCAGTGCT GGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACCG TGGACAAGTCCAAGTGGCAGCAGGGCAACGTGTTACAGCTGC AGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAA GTCCCTGAGCCTGAGCCCCCGCAAG
	LC DNA sequence	GATATTGAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGFGGGCGATAGAGTGACTATCACTGTAGAGCCTCTCAGT

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	(SEQ ID NO:223)	CTATTAGCCTGTGGCTGAACTGGTATCAGCAGAAGCCCGGTAAAGCCCCTAAGCTGCTGATCTACGCCGCTCTACCCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCCTGACTATCTCTAGCCCTGCAGCCCGAGGACT TCGCTACCTACTACTGTCAGCAGTACTACACTAGCCCTTACA CCTTCGGTTCAGGGCACTAAGGTCGAGATTAAAGCGTACGGTG GCCGCTCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCA GCTGAAGAGCGGCACCGCCAGCGTGGTGTGCTGCTGAACA ACTTCTACCCCCGGGAGGCCAAGGTGCAAGTGGAAAGGTGGAC AACGCCCCGAGAGCGGCAACAGCCAGGAGAGCGTCACCG AGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACC CTGACCCCTGAGCAAGGCCGACTACGAGAAGCATAAGGTGTA CGCCTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCGTGA CCAAGAGCTTCAACAGGGGCGAGTGC
NOV0712	LCDR1 (Kabat) (SEQ ID NO:224)	RASQSISSYLN
	LCDR2 (Kabat) (SEQ ID NO:225)	AVSTLQS
	LCDR3 (Kabat) (SEQ ID NO:226)	QSGTTPPTT
	HCDR1 (Kabat) (SEQ ID NO:227)	SHGMH
	HCDR2 (Kabat) (SEQ ID NO:228)	VISGSGSNTGYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:229)	QWGSYAFDS
	vH full sequence (SEQ ID NO:230)	QVQLLESGGGLVQPGGSLRLSCAASGFTFSSHGMHWVRQAPG KGLEWVSVISGSGSNTGYADSVKGRFTISRDN SKNTLYLQMNS LRAEDTAVYYCARQWGSYAFDSWGQGTILVTVSS
	vL full sequence (SEQ ID NO:231)	DIQMTQSPSSLSASVGRVTITCRASQSISSYLNWYQQKPKAP KLLIYAVSTLQSGVPSRFSGSGSDFTLTISLQPEDFATYYCQ QSGTTPPTTFGQGTIKVEIK
	vH DNA sequence (SEQ ID NO:232)	CAGGTGCAGCTGCTGGAATCAGGCCGGCGGACTGGTGCAGCC TGGCGGATCCCTGAGGCTGAGCTGCGCTGCTAGTGGCTTCA CCTTTAGCTCTCACGGAATGCACTGGGTCCGCCAGGCCCTG GTAAAGGCCTCGAGTGGGTGTCAGTGATTAGCGGTAGCGGC TCTAACACCCGGCTACGCCGATAGCGTGAAAGGCCCGGTTCAC TATCTCTAGGGATAACTCTAAGAAACACCCTGTACCTGCAGA TGAACCTCCCTGAGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGACAGTGGGGCTCCTACGCCTTCGATAGCTGGGGTCA

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		AGGCACCCCTGGTTCACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:233)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCTCTAGCTACCTGAACCTGGTATCAGCAGAAGCCCGGT AAAGCCCCFAAGCTGCTGATCTACGCCGTGTCTACCCCTGCA GTCAGGCGTGCCCTCTAGGTTTACGGGTAGCGGTAGTGGCA CCGACTTCACCCCTGACTATTAGTAGCCTGCAGCCCCGAGGAC TTCCGTACCTACTACTGTACGAGTCAGGCACCTTCCCCCCT ACTACCTTCGGTACAGGCACTAAGGTTCGAGATTAAG
	HC full sequence (SEQ ID NO:234)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSSHGMIHWVRQAPG KGLEWVSVISGSGSNTGYADSVKGRFTISRDNSENKNTLYLQMNS LRAEDTAVYYCARQWGSYAFDSWQGILVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVFVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRTPE VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTIVLHQDWLNGKEYECKVSNKALPAPIEKTIKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTIPTPVLDSGFFLYSKLTVDKSRWQQGNVPSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:235)	DIQMTQSPSSLSASVGDRTITTCRASQSISSYLNWYQQKPKAP KLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSGTFPPTTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSFYSLS SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:236)	CAGGTCAGCTGCTGGAATCAGGCCGCGGAGCTGGTGCAGCC TGCGCGATCCCTGAGGCTGAGCTGCCCTGCTAGTGGCTCA CCFTTAGCTCTCACGGAATGCACTGGGTCCGCCAGGCCCTG GTAAAGGCCTCGAGTGGGTGTCACTGATTAGCGGTAGCGGC TCTAACACCGGCTACGCCGATAGCGTGAAGGGCCCGGTTCAC TATCTCTAGGGATAACTCTAAGAACACCCCTGTACCTGCAGA TGAACCTCCCTGAGGGGCCGAGGACACCGCCGTCTACTACTGC GCTAGACAGTGGGGCTCCTACGCCCTTCGATAGCTGGGGTCA AGGCACCCCTGGTCACCGTGTCTAGCGCTAGCACTAAGGGCC CAAGTGTGTTTCCCCCTGGCCCCCAGCAGCAAGTCTACTTCCG GCCGAACTGCTGCCCTGGGTTCCTGGTGAAGGACTACTTC CCCGAGCCCGTGACAGTGTCTCTGGAACCTCTGGGGCTCTGAC TTCCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCG GCCTGTACAGCCTGAGCAGCGTGGTGACAGTGCCTCCAGC TCTCTGGGAACCCAGACCTATATCTGCAACGTGAACCACAA GCCCAGCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAG AGCTGCGACAAGACCCACACCTGCCCCCCTGCCCAAGCTCC AGAACCTGCTGGGAGGGCCTTCCGTGTCTCTGTTCCTCCCCCAA GCCCAGGACACCTGATGATCAGCAGGACCCCGAGGTGA CCTGCGTGGTGGTGGACGTGTCTCCACGAGGACCCAGAGGTG AAGTTCAACTGGTACCTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTAC AGGGTGGTGTCCGTGCTGACCTGTCTGCACCAAGGACTGGCT GAACGGCAAAGAATACAAGTGCAAAGTCTCCAACAAGGCC CTGCCAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAGG GCCAGCCACGGGAGCCCCAGGTGTACACCTGCCCCCCAGC CGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCT

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		GGTGAAGGGCTTCTACCCAGCGATATCGCCGTGGAGTGGG AGAGCAACGGCCAGCCCGAGAACAACACTACAAGACCACCCC CCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCA AGCTGACCGTGGACAAGTECAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACT ACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
	LC DNA sequence (SEQ ID NO:237)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCTCTAGCTACCTGAACCTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGTGTCTACCCCTGCA GTCAGGCGTGCCCTCTAGGTTTACGGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATTAGTAGCCTGCAGCCCGAGGAC TTCGCTACCTACTACTGTTCAGCAGTCAGGCACCTTCCCCCT ACTACCTTCGGTCAGGGCACTAAGGTGAGATTAAGCGTAC GGTGGCCGCTCCAGCGTGTTTCATCTTCCCCCAGCGACGA GCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGA ACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAAGGTG GACAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCA CCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGC ACCCTGACCCCTGAGCAAGGCCGACTACGAGAAGCATAAGGT GTACGCCGTGCGAGGTGACCCACCAGGGCCTGTCCAGCCCCG TGACCAAGAGCTTCAACAGGGGCGAGTGC
NOV0713	LCDR1 (Kabat) (SEQ ID NO:238)	SGDNLRSYVH
	LCDR2 (Kabat) (SEQ ID NO:239)	GNNKRPS
	LCDR3 (Kabat) (SEQ ID NO:240)	GVYTLSSVV
	HCDR1 (Kabat) (SEQ ID NO:241)	SNSAAWN
	HCDR2 (Kabat) (SEQ ID NO:242)	RIYYRSKWYNDYAVSVKS
	HCDR3 (Kabat) (SEQ ID NO:243)	GLVGRYGQPYHFDV
	vH full sequence (SEQ ID NO:244)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRIYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCARGLVGRYGQPYHFDVWQQGTLVTVSS
	vL full sequence (SEQ ID NO:245)	DIELTQPPSVSVSPGQTASITCSGDNLRSYVHVHWYQKPGQAP VLVIYGNNKRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYC GVYTLSSVVFPGGGLTKLTVL

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vH DNA sequence (SEQ ID NO:246)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAAGTGGATTGTCAGA GCCCCAGCCGTGGCTCTGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCGCGCGTGGTCTGGTTGGTCGTTACGGTCAGCCG TACCAATTCGATGTTTGGGGCCAAGGCACCCCTGGTGACTGTT AGCTCA
vL DNA sequence (SEQ ID NO:247)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCACTACCTGTAGCGGCGATAACCTGC GTTCTTACTACGTTTATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGGTGGTGATCTACGGTAACAACAAACGTCGAG CGGCATCCCGGAACGTTTATAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAAGCGGCACCCAGGCGGGAAGACGAA GCGGATTATTACTGCGGTGTTTACACTCTGTCTTCTGTTGIG TTTGGCGGCGGCACGAAGTTAACCGTCCTA
HC full sequence (SEQ ID NO:248)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSNSAAWNWIRQSP SRGLEWLGRYYRSKWNNDYAVSVKSRITINPDYSKNQFSLQL NSVTPEDTAVYYCARGLVGRYGQPYHFDVWGGGTGLTVSSAS TKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKRVPEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMSRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHREALHINHYTQKSLSLSPGK
LC full sequence (SEQ ID NO:249)	DIELTQPPSVSVSPGQFASITCSGDNLSRYVHWYQQKPKQAP VLVIYGNKRPSGIPERFSGSNSONTATLTISGTQAEDEADYYC GVYTLSSVVFQGGTKLTVLGPKAAPSVTLFPPSSEELQANKA TLVCLISDPYPGAVTVAWKADSSPVKAGVETTPPSKQSNNKYA ASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
HC DNA sequence (SEQ ID NO:250)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGCTTGGAAGTGGATTGTCAGA GCCCCAGCCGTGGCTCTGAGTGGCTGGGCCGTATCTACTAC CGTAGCAAATGGTACAACGACTATGCCGTGAGCGTGAAAAAG CCGCATTACCATTAACCCGGATACTTCGAAAAACCAGTTTA GCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGTG TATTATTGCGCGCGTGGTCTGGTTGGTCGTTACGGTCAGCCG TACCAATTCGATGTTTGGGGCCAAGGCACCCCTGGTGACTGTT AGCTCAGCCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCA CCCTCCTCCAAGAGCACCTCTGGGGGACAGCGGCCCTGGG CTGCCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGT CGTGGAAGTCAGGCGCCCTGACCAGCGGCGTGACACACCTTC CCGGCTGTCTACAGTCCTCAGGACTCTACTCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTA CATCTGCAACGTGAATCACAAGCCCAAGCAACACCAAGTTGG ACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTACACA TGCCCAACCGTGCCCAAGCAGCTGAACTCCTGGGGGGACCGTC

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		AGTCTTCTCTTCCCCCAAAACCCAAGGACACCCCTCATGAT CTCCCGAACCCCTGAGGTACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGG AGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCTCACC GTCTGCAACAGGACTGGCTGAATGGCAAGGAGTACAAGTG CAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAA CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG TACACCCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCA GGTCAGCCCTGACCTGCTTGGTCAAAGGCTTCTATCCAGCG ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA CAACTACAAGACCACGCCCTCCGTGCTGGACTCCGACGGCT CCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG TGGCAGCAGGGGAACGTCCTCTCATGCTCCGTGATGCATGA GGCTCTGCACAACCACTACACGCAGAAGAGCCCTCTCCCTGT CTCCGGGTAAA
	LC DNA sequence (SEQ ID NO: 251)	GATATCGAACTGACCCAGCCGCCGAGCGTGACCGTGAGCCC GGGCCAGACCCGCGAGCATTACCTGTAGCGGCGATAACCTGC GTCTTACTACGTTTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGGTAACAACAAACGTCGGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTAATGCGGTGTTTACACTCTGTCTTCTGTTGTG TTTGGCGGCGGCACGAAGTTAACTGCTTAGGTGAGCCCAA GGCTGCCCCCTCGGTCACTCTGTTCCTCCGCTCTCTGAGGA GCTTCAAGCCAAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCTTGAAGGCAAGAT AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCCTC CAAACAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG AGCCTGACGCCCTGAGCAGTGGAAAGTCCCACAGAAGCTACAG CTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCCCTACAGAATGTTCA
NOV0718	LCDR1 (Kabat) (SEQ ID NO: 252)	SGDKIPTTYTVH
	LCDR2 (Kabat) (SEQ ID NO: 253)	DDNKRPS
	LCDR3 (Kabat) (SEQ ID NO: 254)	QSTASGTVV
	HCDR1 (Kabat) (SEQ ID NO: 255)	SYALH
	HCDR2 (Kabat) (SEQ ID NO: 256)	RIKSKTNGGTTDYAAPVKG
	HCDR3 (Kabat)	VDATYSYSGYYYPMDY

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	(SEQ ID NO:257)	
	vH full sequence (SEQ ID NO:258)	QVQLVESGGGLVKPGGSLRLSCAASGFTFNSYALHWVRQAPGKGLEWVVGRIKSKTNGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCARVDATYSYSGYYYPMQYWGQGTILVTVSS
	vL full sequence (SEQ ID NO:259)	DIELTQPPSVSVSPGQTASITCSGDKIPTTYTVHWYQQKPGQAPVLIYYDDNKRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSTASGTVVFGGGTKLTVL
	vH DNA sequence (SEQ ID NO:260)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACCAGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCCTCCGATTCACTTTAACTCTTACGCTCTGCATTGGGTGCGCCAGGCCCGGGCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAACTAACGGTGGTACTACTGACTATGCCGCCCCAGTGAAAGGCCGCTTTACCATTAGCCGCGATGATTCGAAAAACACCCCTGTATCTGCAAATGAACAGCCTGAAAACCGAAGATACGGCCCGTGTATTATTGCGCGCGTGTGACGCTACTTACTCTTACTCTGGTACTACTACCCGATGGATTACTGGGGCCAAGGCACCCCTGGTGACTGTAGCTCA
	vL DNA sequence (SEQ ID NO:261)	GATATCGAACTGACCCAGCCGCGGAGCGTGAGCGTGAGCCC GGCCAGACCGCGAGCATTACCTGTAGCGCGATAAAATCCCGACTTACACTGTTTATTGGTACCAGCAGAAACCGGGCCAGGCGCCGGTGTCTGGTATCTACGACGACAACAAACCTCCGAGCGGCATCCCGGAACGTTTATAGCGGATCCAACAGCGGCAACA CCGGACCCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTACTGCTCTGGTACTGTTGTGTTGGCGCGCGCACGAAGTTAACCTCCTA
	HC full sequence (SEQ ID NO:262)	QVQLVESGGGLVKPGGSLRLSCAASGFTFNSYALHWVRQAPGKGLEWVVGRIKSKTNGGTTDYAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCARVDATYSYSGYYYPMQYWGQGTILVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEPKSCDKHTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTSKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDIGSFYLYSKLTVDKSRWQQGNVFPSCSVMEALHNHYTOKSLSLSPGK
	LC full sequence (SEQ ID NO:263)	DIELTQPPSVSVSPGQTASITCSGDKIPTTYTVHWYQQKPGQAPVLIYYDDNKRPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQSTASGTVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSYLSTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:264)	CAGGTGCAATTGGTGGAAAGCGGCGGTGGCCTGGTGAAACCAGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCCTCCGATTCACTTTAACTCTTACGCTCTGCATTGGGTGCGCCAGGCCCGGGCAAAGGTCTCGAGTGGGTGGGCCGTATCAAATCTAAAACTAACGGTGGTACTACTGACTATGCCGCCCCAGTGAAAGGCCGCTTTACCATTAGCCGCGATGATTCGAAAAACACCCCTGTATCTGCAAATGAACAGCCTGAAAACCGAAGATACGGCCCGTGTATT

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		<p> ATTGCGCGCGTGTGACGCTACTTACTCTTACTCTGGTTACT ACTACCCGATGGATTACTGGGGCCAAGGCACCCTGGTGACT GTTAGCTCAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTG GCACCCCTCTCCAAGAGCACCTCTGGGGGACAGCGGCCCT GGGCTGCCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG TGTCGTGGAATCAGCGGCCCTGACCAAGCGGCGTGACACCC TTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGAC CTACATCTGCAACGTGAATCACAAGCCCAAGCAACACCAAGG TGGACAAGAGAGTTGAGCCCAAAATCTTGTGACAAAATCAC ACATGCCCACCGTGCCCAAGCACCTGAACTCCTGGGGGACCC GTCAGTCTTCTCTTCCCCCAAAAACCAAGGACACCCTCAT GATCTCCCGAACCCCTGAGGTCACATGCGTGGTGGTGGACG TGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTG GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG AGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCTCT ACCGTCTGCAACCAGGACTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGA AAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACACACA GGTGTACACCTGCCCCCATCCCCGGGAGGAGATGACCAAGA ACCAGGTACGCTGACCTGCGCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACTACAAGACCACGCTCTCCGTGCTGGACTCCGAC GGCCTCTCTTCTCTACAGCAAGCTCACCGTGGACAAGAG CAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCC CTGTCTCCGGGFAAA </p>
	LC DNA sequence (SEQ ID NO:265)	<p> GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCAGCGAGCAATACCTGTAGCGGCGATAAAATCC CGACTTACACTGTTTATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACGACAACAAACGTCGAG CGGCATCCCGGAACGTTTATGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTAAGTCCAGTCTACTGCTTCTGGTACTGTTGTG TTTGGCGCGCGCACGAAGTTAACCGTCTAGGTCAAGCCAA GGCTGCCCCCTCGGTCACTCTGTFTCCCGCCCTCCTCTGAGGA GCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCTGGAAGGCAGAT AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCCTC CAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG AGCCTGACGCTTGAAGAGTGGAAAGTCCACAGAAAGCTACAG CTGCCAGGTACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCTACAGAAATGTCA </p>
NOV0719	LCDR1 (Kabat) (SEQ ID NO:266)	RASQSIVSYLN
	LCDR2 (Kabat) (SEQ ID NO:267)	DASSLOS
	LCDR3 (Kabat) (SEQ ID	QQSGSHSIT

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	NO:268)	
	HCDR1 (Kabat) (SEQ ID NO:269)	SHWVH
	HCDR2 (Kabat) (SEQ ID NO:270)	VISYMGSSSTYYADSVKQ
	HCDR3 (Kabat) (SEQ ID NO:271)	GSYDMAFDV
	vH full sequence (SEQ ID NO:272)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSHWVHWVRQAPG KGLEWVSVISYMGSSSTYYADSVKGRFTISRDNKNTLYLQMNS LRAEDTAVYYCARGSYDMAFDVWGQGTLLTVSS
	vL full sequence (SEQ ID NO:273)	DIQMTQSPSSLSASVGDRTVITCRASQSVSYLNWYQQKPGKAP KLLIYDASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSGSHSITFGQGTKVEIK
	vH DNA sequence (SEQ ID NO:274)	CAGGTGCAGCTGCTGGAATCAGGCCGGCGACTGGTGCAGCC TGGCGGTAGCCTGAGACTGAGCTGCGCTGCTAGTGGCTTCA CCTTAGCTCTCACTGGGTGCACTGGGTCAGACAGGCCCTG GTAAAGGCCCTGGAGTGGGTGTCACTGATTAGCTATATGGGC TCTAGCACCTACTACGCCGATAAGCGTGAAGGCCCGGTTAC TATCTCTAGGGATAACTCTAAGAACACCCTGTACCTGCAGA TGAATAGCCTGAGAGCCGAGGACACCGCCCTCTACTACTGC GCTAGAGGCTCCTACGATATGGCCTTCGACGTGTGGGGTCA GGGCACCCTGGTCAACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:275)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCGTCAGCTACCTGAAGTGGTATCAGCAGAAGCCCGGT AAAGCCCTAAGCTGCTGATCTACGACGCCCTCTAGCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCCTGACTATTAGTAGCCTGCAGCCCGAGGAC TTCGCTACCTACTACTGTCAGCAGTCAGGCTCTCACTCTATC ACCTTCGCTCAGGCGCTAAGGTCGAGATTAAG
	HC full sequence (SEQ ID NO:276)	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSHWVHWVRQAPG KGLEWVSVISYMGSSSTYYADSVKGRFTISRDNKNTLYLQMNS LRAEDTAVYYCARGSYDMAFDVWGQGTLLTVSSASTKGPSVF PLAPSSKSTSGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK RVEPKSCDKTHTCPPAPPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNOVSLTCLVKGFYPSDIAVEWESN GQPENNYKTIPTVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:277)	DIQMTQSPSSLSASVGDRTVITCRASQSVSYLNWYQQKPGKAP KLLIYDASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSGSHSITFGQGTKVEIKRTVAAPSVEIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSITYLS

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		STLTLKADYKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:278)	CAGGTGCAGCTGCTGGGAATCAGGCCGCGCGACTGGTGCAGCC TGGCGGTAGCCTGAGACTGAGCTGCGCTGCTAGTGGCTTCA CCTTTAGCTCTCACTGGGTGCACTGGGTGACAGACAGGCCCTG GTAAAGGCCTGGAGTGGGTGTCAGTGATTAGCTATATGGGC TCTAGCACCTACTACGCCGATAGCGTGAAGGGCCGGTTTAC TATCTCTAGGGATAACTCTAAGAACACCTGTACCTGCAGA TGAATAGCCTGAGAGCCGAGGACACCGCCGTCTACTACTGC GCTAGAGGCTCTACGATATGGCCTTCGACGTGTGGGGTCA GGGCACCCGCGTACCGTGTCTAGCGCTAGCACTAAGGGCC CAAGTGTGTTTCCCTGGCCCCCAGCAGCAAGTCTACTTCCG GCGGAACCTGCTGCCCTGGGTGCTGCTGGTGAAGCACTACTC CCCGAGCCCGTGACAGTGTCTTGGAACTCTGGGGCTCTGAC TTCCGGCGTGACACCTTCCCCGCCGTGCTGCAGAGCAGCG GCCTGTACAGCCTGAGCAGCGTGGTGACAGTGCCCTCCAGC TCTCTGGGAACCCAGACCTATATCTGCAACGTGAACCACAA GCCCAGCAACACCAAGGTGGACAAGAGAGTGGAGCCCAAG AGCTGCGACAAGACCCACACCTGCCCCCCCTGCCCCAGCTCC AGAACCTGCTGGGAGGGCCCTTCCGTGTTCTGTTCCTCCCCCAA GCCCCAAGGACACCTGATGATCAGCAGGACCCCCGAGGTGA CTTGGCTGGTGGTGGACGTGTCCCAAGAGGACCCAGAGGTG AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAACAACGC CAAGACCAAGCCAGAGAGGAGCAGTACAACAGCACCTAC AGGGTGGTGTCCGTGCTGACCGTGTCTGACCAAGGACTGGCT GAACGGCAAGAATACAAGTGCAAAAGTCTCCAACAAGGCC CTGCCAGCCCCAATCGAAAAGACAATCAGCAAGGCCAAGG GCCAGCCACGGGAGCCCCAGGTGTACACCTTCCCCCCCAGC CGGGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCT GGTGAAGGGCTTCTACCCAGCGATATCGCGTGGAGTGGG AGAGCAACGGCCAGTCCGAGAACAACTACAAGACTACCCC CCCAGTGTCTGGACAGCGACGGCAGCTTCTTCTGTACAGCA AGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGT GTTCAGCTGCAGCGTGTATGCAAGAGGCCCTGCACAACCACT ACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG
	LC DNA sequence (SEQ ID NO:279)	GATATTGAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGT CTATCGTCAGCTACCTGAACTGGTATCAGCAGAAGCCCGGT AAAGCCCTAAGCTGCTGATCTACGACGCCTCTAGCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGGCA CCGACTTCACCTGACTATTAGTAGCCTGCAGCCCGAGGAC TTGGCTACCTACTACTGTGAGCAGTCAGGCTCTCACTTATC ACCTTCGGTCAGGGCACTAAGGTGAGAGATTAAGCGTACGGT GGCCGCTCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGC AGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCTGCTGAAC AACTTCTACCCCCGGGAGGCCAAGGTGCAAGTGAAGGTGGA CAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACC GAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCTTGAGCAAGGCCGACTACGAGAAGCATAAGGTG TACGCTTGGAGGTGACCCACAGGCGCTGTTCAGCCCCGT GACCAAGAGCTTCAACAGGGGCGAGTGC
NOV0720	LCDRI (Kabat) (SEQ ID NO:280)	SGDNIGSMTAH

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LCDR2 (Kabat) (SEQ ID NO: 281)	DKNERPS
LCDR3 (Kabat) (SEQ ID NO: 282)	QSWDDSYNSVV
HCDR1 (Kabat) (SEQ ID NO: 283)	SNSAGWN
HCDR2 (Kabat) (SEQ ID NO: 284)	RIYYRSK WYNDYAVSVKS
HCDR3 (Kabat) (SEQ ID NO: 285)	EKYTVSFYDFFDY
vH full sequence (SEQ ID NO: 286)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSNSAGWNWIRQSP SRGLEWLGRIYYRSK WYNDYAVSVKSRITINPDTSKNQPSLQL NSVTPEDTAVYYCAREKYTVSPYDFFDYWGQGTLVTVSS
vL full sequence (SEQ ID NO: 287)	DIELTQPPSVSVSPGGTASITCSGDNIGSMTAHWYQQKPGQAPV LVIYDKNERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQ SWDDSYNSVVFGGGTLTVL
vH DNA sequence (SEQ ID NO: 288)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCCCTGAGCCTGACCTGCOCGATTTCCGGAGATA GCGTGAGCTCTAACTCTGCTGGTTGGAAGTGGATTCCGTACG AGCCCGAGCCGTGGCCTCGAGTGCTGGGCCGTATCTACTA CCGTAGCAAAATGGTACAACTACTATGCCGTGAGCGTGAAGAA GCCGCATTACCATTAACCCGGATACTTCGAAAAAACCAGTTT AGCCTGCAACTGAACAGCGTGACCCCGAAGATACGGCCGT GTATTATTGCGCGCGTGAAAAAATACACTGTTTCTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCCTGGTGACTGTAG CTCA
vL DNA sequence (SEQ ID NO: 289)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCC GGGCCAGACCGCGAGCATTACCTGTAGCGCGATAACATCG GTTCTATGACTGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAACGTTTATAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGACTCTTACAACCTT GTTGTGTTTGGCGGCGGCACGAAGTTAACCCTCCTA
HC full sequence (SEQ ID NO: 290)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSNSAGWNWIRQSP SRGLEWLGRIYYRSK WYNDYAVSVKSRITINPDTSKNQPSLQL NSVTPEDTAVYYCAREKYTVSFYDFFDYWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVFPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTL

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		MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTOKSLSLSPGK
	LC full sequence (SEQ ID NO:291)	DIETQPPSVSVSPGQTASITCSGDNIGSMTAHWYQQKPGQAPV LVIYDKNERPSGIPERFSGSNSGNTATLTISGTQAEDEADYYCQ SWDDSYNSVVPGGGTKLTVLQPKAAPSVTLFPPSSEELQANK ATLVCLISDFYPGAVTVAWKADSSPVKAGVETITPPSKQSNMKY AASSVLSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:292)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCCCTGGTGAAACC GAGCCAGACCCCTGAGCCTGACCTGCGCGATTTCGGGAGATA GCGTGAGCTCTAACTCTGCTGGTTGGAACCTGGATTCCGTCAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTA CCGTAGCAAATGGTACAAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAAACAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTGAAAAATACTGTTTCTTCTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCCTGGTGACTGTTAG CTCAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTGGCACC CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT GCCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTG TGGAACTCAGGCGCCCTGACCAGCGCGGTGCACACCTTCCC GGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGT GGTGACCGTGCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCCAGCAACACCAAGGTGGAC AAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACACATG CCCACCGTGCCAGCACCTGAACCTCTGGGGGACCGTCAO TCTTCTCTTCCCCCCTCAAAACCAAGGACACCCCTCATGATCT CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGC CACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG CAGTACAACAGCACGTACCGGGTGGTCAAGCTTCAACCGT CCTGCAACAGGACTGGCTGAATGGCAAGGAGTACAAGTGCA AGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAAACC ATCTCCAAGGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA CACCTTGCCCCCATCCCCGGGAGGAGATGACCAAGAACCAGG TCAGCCTGACCTGCCCTGGTCAAAGGCTTCTATCCCAGCGAC ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA ACTACAAGACCACGCCTCCCGTGGTGGACTCCGACGGCTCC TTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTG GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGATGAGG CTCTGCACAACCACTACACGCAGAAAGAGCCTCTCCCTGTCTC CGGGTAAA
	LC DNA sequence (SEQ ID NO:293)	GATATCGAACTGACCCAGCCGCCGAGCGTGAGCGTGAGCCCC GGGCCAGACCGCGAGCAATTACCTGTAGCGGCGGATAACATCG GTTCTATGACTGCTCAATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGTCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAACGTTTACCGGATCCAACAGCGGCAACA CCGGCAGCCCTGACCATTAAGCGGCACCCAGGCGGAAGACGAA GCGGATTATTACTGCCAGTCTTGGGACGACTCTTACAACCTCT GTTGTGTTTGGCGGCGGCACGAAAGTTAACCCTCTAGGTCA GCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCTCTC TGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGCTCTCA

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	TAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAG GCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA CACCCCTCCAAACAAAGCAACAACAAGTACGCGCCAGCAG CTATCTGAGCCTGACGCCTGAGCAGTGGGAAGTCCCACAGAA GCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG AAGACAGTGGCCCCCTACAGAAATGTTCA
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Example 2: Antibody engineering by site-directed mutagenesis

[00381] Site-directed mutagenesis for the removal of the potential sites for post-translational modifications and germlining was performed for a subset of anti-CDH6 antibodies using the QuikChange® Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA). NOV690 sequence was changed D₃₃S and VL (3-J-germline) resulting in antibody NOV1126. NOV695 sequence was changed N₃₃Q and S₄₀Y, resulting in antibody NOV1127. NOV0720 sequence was changed N₃₃Q, N₄₀Q and VL (3-J-germline), resulting in antibody NOV1132. Sequence information for the engineered antibodies is described in Table 6.

Table 6*Sequence information of engineered antibodies*

NOV1126	LCDR1 (Kabat) (SEQ ID NO:294)	SGDAIGTKFAH
	LCDR2 (Kabat) (SEQ ID NO:295)	YDHERPS
	LCDR3 (Kabat) (SEQ ID NO:296)	YSRASSNLV
	HCDR1 (Kabat) (SEQ ID NO:297)	DHAID
	HCDR2 (Kabat) (SEQ ID NO:298)	VIAGSGSITYYADSVKG
	HCDR3 (Kabat) (SEQ ID NO:299)	DTGVYREYMDV
	vH full sequence	EVQLLESGGGLVQPGGSLRLSCAASGFTFS DHAIDWVRQAPGK GLEWVSVIAGSGSITYYADSVKGRFTISRDN SKNTLYLQMNSL

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(SEQ ID NO:300)	RAEDTAVVYCARDTGVYREYMDVWVGQGLVTVSS
vL full sequence (SEQ ID NO:301)	SYELTQPLSVSVALGQTARITCSGDAIGTKFAHWYQOKPGQAP VLVIYYDHERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC YSRASSNLVFGGGTKLTVL
vH DNA sequence (SEQ ID NO:302)	GAAGTGC AATTGCTGGAAAGCGGGGGTGGCCGTGGTGCAGCC GGGTGGCAGCCTGCCTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTGACCATGCTATCGACTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTTTCGGTATCGCTGGTAGCGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCCGCTTTACC ATCAGCCGCGATAATTGCAAAAACACCCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATAACGGCCGTGTATTATTGCG CGCGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCCTGGTGACTGTTAGCTCA
vL DNA sequence (SEQ ID NO:303)	AGCTATGAACTGACCCAGCCGCTGAGCGTGAGCGTGCGCCT GGGCCAGACCGCGCGCAATTACCTGTAGCGCGATGCTATCG GTACTAAATTCGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATAGCCGCGCGCAGGCGGGCGACGAA GCGGATTATTACTGCTACTCTCGTGCTTCTTCTAACCTGGTG TTTGGCGGCGGCACGAAAGTTAACCGTCTCA
HC full sequence (SEQ ID NO:304)	EVQLLESQGGGLVQPGGSLRLSCAASGFTFSDAIDWVRQAPGK GLEWVSVIAGSGSITYYADSVKGRFTISRDNKNTLYLQMNSL RAEDTAVVYCARDTGVYREYMDVWVGQGLVTVSSASTKGPS VFPLAPSSKSTSGGTAALQCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHFTCPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQV NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTFPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
LC full sequence (SEQ ID NO:305)	SYELTQPLSVSVALGQTARITCSGDAIGTKFAHWYQOKPGQAP VLVIYYDHERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC YSRASSNLVFGGGTKLTVLGGPKAAPSVTLPFPSSSEELQANKA TLVCLISDFYPGAVTVAWKADSSPVKAQVETTTTPSKQSNKYA ASSYLSTPEQWKSHRSYSQCVTHEGSTVEKTVAPTECS
HC DNA sequence (SEQ ID NO:306)	GAAGTGC AATTGCTGGAAAGCGGGGGTGGCCGTGGTGCAGCC GGGTGGCAGCCTGCCTCTGAGCTGCGCGGCGTCCGGATTCA CCTTTTCTGACCATGCTATCGACTGGGTGCGCCAGGCCCGG GCAAAGGTCTCGAGTGGGTTTCGGTATCGCTGGTAGCGGTT CTATCACCTACTATGCGGATAGCGTGAAAGGCCCGCTTTACC ATCAGCCGCGATAATTGCAAAAACACCCCTGTATCTGCAAAT GAACAGCCTGCGTGCGGAAGATAACGGCCGTGTATTATTGCG CGCGTGACACTGGTGTTTACCGTGAATACATGGATGTTTGG GGCCAAGGCACCCCTGGTGACTGTTAGCTCAGCCTCCACCAA GGGTCCATCGGCTTCCCCCTGGCACCCCTCCTCCAAGAGCAC CTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT ACTTCCCCGAACCGGTGACGGGTGTCTGTGAACCTCAGGCGCC CTGACCAGCGCGCTGCACACCTTCCCCGCTGTCTACAGTCC

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		TCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCA CAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCC AAATCTGTGTGACAAAACCTACACATGCCCAACCGTGCCAGC ACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCC'CCCC AAAACCCAAAGGACACCCCTCATGATCTCCCGGACCCCTGAGG TCACATCCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG TACCGGGTGGTACGCGTCTCACCGTCTGACACAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA GCCCTCC'AGCC'CCCATCGAGAAAACCATCTCCAAGCCAA AGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCAT CCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGAGAGAACAACCTACAAGACCAG CCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACG TCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
	LC DNA sequence (SEQ ID NO:307)	AGCTATGAACTGACCCAGCCGCTGAGCGTGAACGTGGCGCT GGGCCAGACCGCGCGCATTACCTGTAGCGGCGATGCTATCG GTACTAAATTCGCTCATTTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGTGGTGTCTACTACGACCATGAACGTCCGAG CGGCATCCCGGAACGTTTATGCGGATCCAACAGCGGCAACA CCGCGACCTGACCATTAGCCGCGCGCAGGCGGGCGACGAA GCGGATTATTACTGCTACTCTCGTGTCTTCTTAACCTGGTG TTGCGCGCGGCACGAAGTTAACCCTCCTAGGTCAOCCCAA GGCTGCCCCCT'GGTCACTCTGT'TCCCGCCCTCCTCTGAGGA GCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG ACTTCTACCCGGGAGCCGTGACAGTGGCTGGAAAGGCAGAT AGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCTC CAAACAAAGCAACAACAAGTACCGCGGCCAGCAGCTATCTG AGCCTGACCCCTGAGCAGTGGAAGTCCACAGAAAGCTACAG CTGCCAGGTACGCATGAAGGGAGCACCGTGGAGAAGACA GTGGCCCCCTACAGAATGTTCA
NOV1127	LCDR1 (Kabat) (SEQ ID NO:308)	RASQGIFTYLN
	LCDR2 (Kabat) (SEQ ID NO:309)	AASTLQS
	LCDR3 (Kabat) (SEQ ID NO:310)	QQYYSTSLT
	HCDR1 (Kabat) (SEQ ID NO:311)	SQSAAWN
	HCDR2 (Kabat)	RIYYRSKWYNDYAVSVKS

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	(SEQ ID NO:312) HCDR3 (Kabat) (SEQ ID NO:313)	ERSYRDYFDY
	vH full sequence (SEQ ID NO:314)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSQSAAWNWIQSP SRGLEWLGRIYYRSK WYNDYAVSVKSRITINPDTSKNQPSLQL NSVTPEDTAVYYCARERSYRDYFDYWGQGTLVTVSS
	vL full sequence (SEQ ID NO:315)	DIQMTQSPSSLSASVGDRTVITCRASQGIFFYLNWYQOKPGKA PKLLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYSTSLTFGQGTKVEIK
	vH DNA sequence (SEQ ID NO:316)	CAGGTGCAGCTGCAGCAGTCAGGCCCTGGCCTGGTCAAGCC TAGTCAGACCCCTGAGCCTGACCTGCGCTATTAGCGGCGATA GTGTGTCTAGTCAGTCAGCCGCTGGAAGTGGATTAGACAG TCACCCCTCTAGGGGCTGGAGTGGCTGGGTAGAATCTACTA TAGGTCTAAGTGGTATAACGACTACGCCGTCAGCGTGAAGT CTAGGATCACTATTAACCCCGACACCTCTAAGAATCAGTTTA GCCTGCAGCTGAATAGCGTGACCCCGAGGACACCGCCGTC TACTACTGCGCTAGAGAGCGGTCCTATAGAGACTACTTCGA CTACTGGGGTCAGGGCACCCCTGGTCACCGTGTCTAGC
	vL DNA sequence (SEQ ID NO:317)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGG GAATCTTCACCTACCTGAAGTGGTATCAGCAGAAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGCTCTACCCCTGCA GTCAGGCGTGCCCTCTAGGTTTAGCGGTAGCGGTAGTGCA CCGACTTCACCCCTGACTATCTCTAGCCCTGCAGCCCGAGGACT TCGCTACCTACTACTGTCAGCAGTACTACTCTACTAGCCTGA CCTTCGCTCAGGGCACTAAGGTGAGATTAAG
	HC full sequence (SEQ ID NO:318)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSQSAAWNWIQSP SRGLEWLGRIYYRSK WYNDYAVSVKSRITINPDTSKNQPSLQL NSVTPEDTAVYYCARERSYRDYFDYWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK VDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDQVEVHNAKTKPREEQ YNSTYRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPOVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
	LC full sequence (SEQ ID NO:319)	DIQMTQSPSSLSASVGDRTVITCRASQGIFFYLNWYQOKPGKA PKLLIYAASLTQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQYYSTSLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSFYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	HC DNA sequence (SEQ ID NO:320)	CAGGTGCAGCTGCAGCAGTCAGGCCCTGGCCTGGTCAAGCC TAGTCAGACCCCTGAGCCTGACCTGCGCTATTAGCGGCGATA GTGTGTCTAGTCAGTCAGCCGCTGGAAGTGGATTAGACAG TCACCCCTCTAGGGGCTGGAGTGGCTGGGTAGAATCTACTA TAGGTCTAAGTGGTATAACGACTACGCCGTCAGCGTGAAGT

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		CTAGGATCACTATTAACCCCGACACCTCTAAGAATCAGTTTA GCCTGCAGCTGAATAGCGTGACCCCGAGGACACCGCCGTC TACTACTGCGCTAGAGAGCGGTCTATAGAGACTACTTCGA CTACTGCGGTACAGGGCACCTTGGTCACCGTGTCTAGCGCTA GCACTAAGGGCCCAAGTGTGTTTCCCTGGCCCCCAGCAGC AAGTCTACTTCCGGCGGAAGTGTGCTGCCCTGGGTGCGTGGT AAGGACTACTTCCCGAGCCCGTGACAGTGTCTGGAACTC TGGGGCTCTGACTTCCGGCGTGCACACCTTCCCCGCCGTGCT GCAGAGCAGCGGCTGTACAGCCTGAGCAGCGTGTGTGACAG TGCCCTCCAGCTCTCTGGGAACCCAGACCTATATCTGCAACG TGAACCAAGCCCAAGCAACACCAAGGTGGACAAGAGAGT GGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCGCCCT GCCCAGCTCCAGAACTGCTGGGAGGGCCTTCCGTGTTCTGT TCCCCCCTAAGCCCAAGGACACCTTGATGATCAGCAGGACC CCCGAGGTGACCTGCCGTGGTGGTGGACGTGTCCACAGGGA CCCAGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGG TGCACAACGCCAAGACCAAGCCCAAGAGAGGAGCAGTACAA CAGCACCTACAGGGTGGTGTCCGTGCTGACCGTGTGCAAC AGGACTGGCTGAACGGCAAAGAATACAAGTGCAAAGTCTCC AACAAGGCCCTGCCAGCCCCAATCGAAAAGACAATCAGCA AGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCCTG CCCCCAGCCGGGAGGAGATGACCAAGAACCAGGTGTCCCT GACCTGTCTGGTGAAGGGCTTCTACCCAGCGATATCGCCG TGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAATAACAA GACCACCCCCCAGTGTCTGGACAGCGACGGCAGCTTCTTCC TGTACAGCAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAG GGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCCTGCA CAACCACTACACCCAGAAAGTCCCTGAGCCTGAGCCCCGGCA AG
	LC DNA sequence (SEQ ID NO:321)	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAG TGTGGGCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGG GAATCTTCACCTACCTGAAGTGGTATCAGCAGAAGCCCGGT AAAGCCCCTAAGCTGCTGATCTACGCCGCCCTCTACCCCTGCA GTCAGGCGTGCCCTCTAGGTTTACCGGTAGCGGTAGTGGA CCGACTTCACCTGACTATCTCTAGCCTGCAGCCCGAGGACT TCGCTACCTACTACTGTTCAGCAGTACTACTCTACTAGCCTGA CCTTCGGTCAAGGGCACTAAGGTCCAGATTAAGCGTACGGTG GCCGCTCCCAAGCGTGTTCATCTTCCCCCAGCGACGAGCA GCTGAAGAGCGGCACCGCCAGCGTGGTGTGCTGTGCTGAACA ACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGAC AACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCG AGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACC CTGACCTGAGCAAGGCCGACTACGAGAAAGCATAAGGTGTA CGCCTGCGAGGTGACCCACCAAGGGCCTGTCCAGCCCCGTGA CCAAGAGCTTCAACAGGGGCGAGTGC
NOV1132	LCDR1 (Kabat) (SEQ ID NO:322)	SQDNIGSMTAH
	LCDR2 (Kabat) (SEQ ID NO:323)	DKNERPS
	LCDR3	QSWDDSYTSVV

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(Kabat) (SEQ ID NO:324)	
HCDR1 (Kabat) (SEQ ID NO:325)	SQSAGWN
HCDR2 (Kabat) (SEQ ID NO:326)	RIYYRSK WYNDYAVSVKS
HCDR3 (Kabat) (SEQ ID NO:327)	EKYTVSFYDFFDY
vH full sequence (SEQ ID NO:328)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSQSAGWNWIRQSP SRGLEWLGRYYRSK WYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCAREKYTVSFYDFFDYWGQGTLVTVSS
vL full sequence (SEQ ID NO:329)	SYELTQPLSVSVALGQITARITCSGDNIGSMTAHWYQQKPGQAP VLVIYDKNERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC QSWDDSYTSVVFQGGTKLTVL
vH DNA sequence (SEQ ID NO:330)	CAGGTGCAATTGCAGCAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCCTGAGCCTGACCTGCGCGATTTCGGAGATA GCGTGAGCTCTCAGTCTGCTGGTTGGAACCTGGATTCGTACAG AGCCCGAGCCGTGGCCTCGAGTGGCTGGGCCGTATCTACTA CCGTAGCA AATGGTACAAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACGGCCGT GTATTATTGCGCGCGTGAAAAATACTGTTTCTTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCCTGGTGACTGTTAG CTCA
vL DNA sequence (SEQ ID NO:331)	AGCTATGAACTGACCCAGCCGCTGAGCGTGAGCGTGCGCGCT GGGCCAGACCGCGCGCAATTACCTGTAGCGCGGATAACATCG GTTCTATGACTGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAAUGTTTTAGCGGATCCAACAGCGGCAACA CCGCGACCCTGACCATTAGCCGCGCGCAGGCGGGCGACGAA GCGGATTATTACTGCCAGTCTTGCGACGACTCTTACACCTCT GTTGTGTTTGGCGGCGGCACGAAGTTAACCCTCCTA
HC full sequence (SEQ ID NO:332)	QVQLQQSGPGLVKPSQTLSTCAISGDSVSSQSAGWNWIRQSP SRGLEWLGRYYRSK WYNDYAVSVKSRITINPDTSKNQFSLQL NSVTPEDTAVYYCAREKYTVSFYDFFDYWGQGTLVTVSSAST KGPSVPEPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVPEPKSCDKTHTCPPCPAPELLGGPSVFLFPPPKPDTL MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN VFSCSVMEALHNHYTQKSLSLSPGK

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LC full sequence (SEQ ID NO:333)		SYELTQPLSVSVALGQTARITCSGDNIGSMTAHWYQQKPGQAP VLVIYDKNERPSGIPERFSGSNSGNTATLTISRAQAGDEADYYC QSWDDSYTSVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNK YAASSYLSLTPEQWKSHRSYSCOVTHEGSTVEKTVAPTECS
	HC DNA sequence (SEQ ID NO:334)	CAGGTGCAATTGCACGACAGAGCGGTCCGGGCTGGTGAAACC GAGCCAGACCCCTGAGCCTGACCTGCGCGATTTCGGAGATA GCGTGAGCTCTCAGTCTGCTGGTTGGAACCTGGATTCTGTCAG AGCCCGAGCCCTGGGCTCGAGTGGCTGGGCGCTATCTACTA CCGTAGCAAATGGTACAAACGACTATGCCGTGAGCGTGAAAA GCCGCATTACCATTAACCCGGATACTTCGAAAAACCAAGTTT AGCCTGCAACTGAACAGCGTGACCCCGGAAGATACCGCCGT GTATTATTGCGCGCGTGAAAAATACACTGTTTCTTTCTACGA CTTCTTCGATTACTGGGGCCAAGGCACCCCTGGTGACTGTTAG CTCAGCCTCCACCAGGGTCCATCGGTCTTCCCCCTGGCAACC CTCCTCCAAGAGCACCTCTGGGGGACAGCGGCCCTGGGCT GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG TGGAACTCAGGCGCCCTGACCAGCGCGTGACACACTTCCC GGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGT GGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATG CCCAACCGTGCCCAAGCACCTGAACCTCCTGGGGGACCCGTAG TCTTCTCTTCCCCCAAAACCCAAAGGACACCCCTCATGATCT CCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGC CACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGG CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG CAGTACAACAGCACGTACCGGGTGGTCAAGCGTCTCACCCT CTTGCAACAGGACTGGCTGAATGGCAAGGAGTACAAGTGA AGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACC ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA CACCTTCCCCCATTCGGGGAGGAGATGACCAAGAAACCTAGG TCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGAC ATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGACA ACTACAAGACCAAGCCCTCCCGTGGTGGACTCCGACGGCTCC TTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTG GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGG CTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTC CGGGTAAA
LC DNA sequence (SEQ ID NO:335)		AGCTATGAACTGACCCAGCCGCTGAGCGTGAGCGTGCGGCT GGGCCAGACCGCGCGCATTACCTGTAGCGGCGATAACATCG GTTCTATGACTGCTCATTGGTACCAGCAGAAACCGGGCCAG GCGCCGGTGCTGGTGATCTACGACAAAAACGAACGTCCGAG CGGCATCCCGGAACGTTTATGCGGATCCAACAGCGGCAACA CCGCGACCCCTGACCATTAGCCGCGCGCAGGCGGGGCGACGAA GCGGATTATTAAGTGGCAGTCTTGGGACGACTCTTACACCTCT GTGTGTGTGGGCGCGGACGAAGTTAACCCTCTAGGTCA GCCCAAGGCTGCCCTCTGGTCACTCTGTTCCTGCCCTCTCTC TGAGGAGCTTCAAGCCAAACAGGCCACACTGGTGTGTCTCA TAAGTGACTTCTACCCGGGAGCGGTGACAGTGGCCTGGAAG GCAGATAGCAGCCCGTCAAGGCCGGAGTGGAGACCACCA CACCTTCCAAACAAAGCAACAACAAGTACCGCGCCAGCAG CTATCTGAGCCTGACGCTGAGCAGTGGAAAGTCCACAGAA GCTACAGCTGCCAAGTCAAGCATGAAGGGAGCACCGTGGAG

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AAGACAGTGGCCCCTACAGAATGTTC

Example 3: Determination of anti-CDH6 IgG binding to cells featuring expression of CDH6 from human, cynomolgus, rat and mouse origins

[00382] To determine anti-CDH6 binding to cells featuring expression of CDH6 from different species, FACS (Fluorescence Activated Cell sorting) analysis was performed on OVCAR3 cells, which express CDH6 endogenously, as well as on CHO cells engineered to express CDH6 from human, cynomolgus, rat and mouse origins.

[00383] OVCAR3 (ovarian serous carcinoma) were obtained from ATCC (#HTB-161). The generation of the CHO cell lines featuring CDH6 expression is described in Example 1 above.

[00384] A cell suspension was prepared by treating cells in culture with Accutase® Cell Dissociation Reagent (#A1110501 Gibco, Grand Island, NY) according to the manufacturer's instructions followed by washing the cells in FACS buffer (PBS/1%BSA, Gibco). Cells were resuspended in FACS buffer at 1×10^6 cells/ml and aliquoted into a 96-well round bottom plate (Corning #CLS3360 at 100µl/well. An 8-point, 1:5 serial dilution of the primary antibodies was prepared to yield a highest final starting concentration of 10 µg/ml and 100µl/well were added to the cell suspension. As a negative control antibody human isotype control IgG (R&D systems #1-001-A Minneapolis, MN) was used. Cells were incubated with the primary antibody solutions for 30 minutes on ice, followed by three washes in 200µl of cold FACS buffer. The cells were resuspended in 100µl of PE-conjugated anti-human Human Fc used at 1/500 dilution (Jackson Immuno Research #109-116-098 West Grove, PA) and cells were incubated for 30 minutes on ice. Following three washes in 200µl cold FACS buffer, cells were analyzed on a BD FACS Canto II® (BD Biosciences, San Jose, CA). Geomean of signal per sample was determined using FlowJo® software and EC50s were determined by plotting the geomean of signal versus concentration and non-linear regression curve fitting using Tibco Spotfire® (Tibco, Boston, MA). All of the 20 analyzed anti-CDH6 antibodies displayed dose-dependent, target-specific binding on OVCAR3 cells (Figure 1) and CHO cells expressing CDH6. No reactivity was observed on wild-type CHO cells, which do not express CDH6. EC50 values are summarized in Table 7.

Table 7

Cellular binding affinity (EC50 [nM]) of CDH6 IgGs on OVCAR3 cells and CHO cells negative for CDH6 expression (CHO-TREX) or engineered to express human, cynomolgus, rat or mouse CDH6.

Antibody	OVCAR3	CHO-cvCDH6	CHO-huCDH6	CHO-moCDH6	CHO-ratCDH6	CHO-TREX
hIgG	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

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NOV0670	4.092	0.281	0.205	0.312	0.521	n.a.
NOV0672	7.524	0.294	0.339	0.525	0.53	n.a.
NOV0674	13.398	0.32	0.494	0.527	0.537	n.a.
NOV0682	17.028	0.624	0.556	0.758	0.538	n.a.
NOV0685	5.214	0.178	0.179	0.23	0.321	n.a.
NOV0689	0.396	0.116	0.257	0.177	0.12	n.a.
NOV0690	0.924	0.777	0.136	3.041	16.595	n.a.
NOV0691	1.056	0.042	0.493	0.104	0.102	n.a.
NOV0692	1.056	0.08	0.107	0.221	0.155	n.a.
NOV0693	4.686	0.528	0.556	0.66	0.55	n.a.
NOV0695	2.64	0.306	0.267	0.427	0.659	n.a.
NOV0699	1.188	1.254	0.972	1.554	1.43	n.a.
NOV0705	0.858	0.005	0.511	0.018	0.646	n.a.
NOV0709	1.518	0.53	n.a.	0.669	0.531	n.a.
NOV0710	0.858	0.15	0.151	0.22	0.152	n.a.
NOV0712	1.122	0.202	0.21	0.537	0.523	n.a.
NOV0713	11.22	4.779	3.115	7.034	8.369	n.a.
NOV0718	0.66	0.313	0.187	0.52	0.322	n.a.
NOV0719	1.914	0.171	0.248	0.527	0.222	n.a.
NOV0720	0.66	0.543	0.428	0.338	0.493	n.a.

Example 4: Determination of binding affinity and cross-reactivity to recombinant CDH6 from human, cynomolgus, rat and mouse by Biacore measurement

[00385] Affinity of the antibodies to recombinant CDH6 protein from human, cynomolgus, rat and mouse origin was determined using SPR technology on a Biacore® T100 instrument (GE Healthcare, Pittsburgh, PA) and with CM5 sensor chips.

[00386] Briefly, HBS-P (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 0.005% Surfactant P20) supplemented with 0.5% Bovine Albumin Fraction V (7.5% solution) (Gibco 15260-037) was used as the running buffer for all the experiments. The immobilization level and analyte interactions were measured by response unit (RU). Pilot experiments were performed to test and confirm the feasibility of the immobilization of the anti-human Fc antibody (Jackson ImmunoResearch 109-006-098 West Grove, PA) and the capture of the test antibodies.

[00387] For kinetic measurements, the experiments were performed in which the antibodies were captured to the sensor chip surface via the immobilized anti-human Fc antibody and the ability of the CDH6 proteins to bind in free solution was determined. Briefly, 30 µg/ml of anti-human Fc antibody at pH 5 was immobilized on a CM5 sensor chip through amine coupling at flow rate of 12 µl/minute on all four flow cells to reach 7500 RU's. 5-10 µg/ml of test antibodies were then injected at 10 µl/min for 12 seconds to flow cell 2,3 and 4. Subsequently, 0.78-50 nM of CDH6 receptor

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extracellular domains (ECD) were diluted in a 2-fold series and injected at a flow rate of 80 μ l/min for 100 seconds over reference (flow cell 1) and test flow cells 2,3 and 4. Table of tested ECDs is listed below. Dissociation of the binding was followed for 10 minutes. After each injection cycle, the chip surface was regenerated with 10 mM Glycine pH2.0 at 60 μ l/min for 30 s. All experiments were performed at 25 °C and the response data were globally fitted with a simple 1:1 interaction model using Biacore® T100 evaluation software version 2.0.3 to obtain estimates of on rate (k_{on}), off-rate (k_{off}) and affinity (K_D). The results suggest most of the CDH6 antibodies assayed in this panel are cross-reactive to human, cynomolgus, rat and mouse (Table 8).

Table 8

Binding affinity of anti-CDH6 IgGs to recombinant CDH6 from human, cynomolgus, rat and mouse by Biacore measurement.

Antibody	KD [M] human CDH6	KD [M] cynomolgus CDH6	KD [M] rat CDH6	KD [M] mouse CDH6
NOV0670	9.329E-09	7.378E-09	7.232E-09	1.903E-08
NOV0672	2.463E-09	4.589E-09	3.291E-09	4.283E-09
NOV0674	1.954E-09	6.995E-09	2.829E-09	4.77E-09
NOV0682	6.188E-09	1.273E-08	5.554E-09	1.776E-07
NOV0685	4.551E-09	4.115E-09	4.636E-09	5.321E-09
NOV0689	5.648E-09	6.402E-09	6.083E-09	8.854E-09
NOV0690	6.969E-09	n/a	n/a	1.111E-09
NOV0691	8.343E-09	9.797E-09	4.332E-09	n/a
NOV0692	5.082E-09	5.554E-09	2.502E-09	2.494E-09
NOV0693	1.184E-09	1.966E-09	1.078E-09	1.843E-09
NOV0695	3.023E-12	n/a	n/a	1.67E-11
NOV0705	2.479E-09	n/a	2.334E-09	4.926E-11
NOV0709	8.174E-10	1.983E-09	6.977E-10	2.139E-09
NOV0710	2.065E-09	3.84E-09	2.385E-09	4.009E-09
NOV0712	2.904E-09	3.784E-09	5.719E-09	4.835E-09
NOV0713	1.419E-08	9.049E-09	7.147E-09	7.006E-08
NOV0718	5.455E-09	6.903E-09	6.608E-09	5.941E-09
NOV0719	1.899E-09	4.944E-09	8.789E-09	5.172E-09
NOV0720	2.47E-09	3.335E-09	1.406E-09	2.268E-09

Example 5: Epitope binning by Biacore

[00388] Epitope Mapping of CDH6 antibodies was determined using Surface Plasmon Resonance (SPR) technology on a Biacore® A-100 instrument (GE Healthcare, Pittsburgh, PA) with a CM5 (s) sensor chip. Briefly, HBS-EP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM,

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EDTA, 0.005% Surfactant P20 supplemented with 0.25% BSA and 10mM calcium) was used as the running buffer for all the experiments. The immobilization level and analyte interactions were measured by response unit (RU). Pilot experiments were performed to test and confirm the feasibility of the immobilization of the anti-human Fc antibody (Catalog number Jackson Immuno Research 109-006-098 West Grove, PA) and the capture of the test antibodies.

[00389] For epitope mapping, anti-human Fc antibody was immobilized on spot 1,2 and 4,5 of all four flow cells at 10,000RU. Spot 5 was used as reference. Each of the primary testing CDH6 antibodies was then captured via the immobilized anti-human Fc on the biosensor chip at a response level greater than 300 RU, followed by injection of the CDH6 protein in spot 1 and 5 at binding levels above 20 RU. Spots 2 and 4 were used as additional reference surface. All five spots of each flow cell were blocked with two injections of human IgG at 1 mg/ml to block any free binding sites of anti-human Fc. Each of the secondary testing antibodies was then injected over spots 1,2 or 4,5 to assess the degree of binding to the complex of primary testing antibody and CDH6 protein. Human IgG isotype control was used as primary and secondary testing negative control antibody. The antibodies were tested in parallel in all four flow cells until all possible combinations of antibody pairs had been evaluated. Regeneration of all flow cell surfaces after each primary and secondary antibody binding cycle was done with injection of 10mM glycine pH2.0.

[00390] The results were evaluated using the epitope mapping module in the Biacore® 4000 evaluation software and presented as a matrix, with primary antibodies in rows and secondary in columns (Figure 2). Additional binding of the secondary testing antibody to the complex of the primary testing antibody and CDH6 protein is indicated by positive response values. Negative pairs are marked and compare to human IgG isotype control. The matrix is then used to map which antibodies recognize which epitopes based on how they can form pairs with other antibodies. Antibody NOV0710 and NOV0712 compete with each other indicating they recognize and overlapping epitope on the antigen. Antibody NOV1127 shows positive binding when it was used as primary and secondary binding antibody, suggesting it may bind to multiple sites on CDH6 or that initial binding alters the conformation and opens up additional binding sites. NOV0719, NOV0692, NOV1126 and NOV1132 all appear to bind distinct epitopes based on this analysis.

Example 6: Epitope mapping by hydrogen-deuterium exchange/mass spectrometry

[00391] Hydrogen-deuterium exchange (HDx) in combination with mass spectrometry (MS) (Woods et al., J. Cell Biochem. 2001 537: 89-98)) was used to map the binding site of antibodies NOV1127, NOV0719, NOV0710 and NOV0712 on the full-length extra-cellular domain (ECD) of hCDH6 full-length (aa54-615), which includes cadherin domains (EC) 1 through 5 (Table 9). In addition, HDx was also performed on a truncated ECD consisting of EC 1 through 3 using antibodies NOV0710 and NOV0712 (Table 10). In HDx exchangeable amide hydrogens of proteins are replaced

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by deuterium. This process is sensitive to protein structure/dynamics and solvent accessibility and, therefore, able to report on locations that undergo a decrease in deuterium uptake upon ligand binding. The goal of these experiments was to identify the potential epitopes and understand the dynamics of hCDH6 when bound to our therapeutic antibodies. It is important to note that changes in deuterium uptake are sensitive to both direct binding and allosteric events; in order to precisely determine the epitope HDx has to be combined with orthogonal technologies (e.g. X-ray crystallography).

[00392] Automated HDx/MS experiments were performed using methods similar to those described in the literature (Chalmers et al., Anal Chem. 2006: 78(4):1005-14). The experiments were performed on a Waters HDx-MS® platform, which includes a LEAP autosampler, nanoACQUITY UPLC System, and Synapt G2 mass spectrometer (Waters Corp, Milford MA). The deuterium buffer used to label the protein backbone of the full length hCDH6(54-615) with deuterium was 50 mM D-Tris, 150 mM NaCl pH 7.4 + 3 mM CaCl₂; the overall percentage of deuterium in the solution was 89.5%. For hCDH6(54-615) deuterium labeling experiments in the absence of hCDH6 antibody, 600 pmol of hCDH6(54-615), volume of 5 µl, was diluted using 45 µl of the deuterium buffer in a chilled tube and incubated for 25 minutes on a rotator at 4 °C. The labeling reaction was then quenched with 75 µl of chilled quench buffer on ice for three minutes followed by injected onto the LC-MS system for automated pepsin digestion and peptide analysis. For hCDH6(54-615) deuterium labeling experiments in the presence of bound hCDH6 antibody, 600 pmol of the hCDH6 antibody was first immobilized on Thermo Protein G Plus beads and cross-linked using disuccinimidyl suberate (DSS). To perform the labeling experiments, the antibody beads (containing 600 pmol antibody) were incubated with 600 pmol hCDH6(54-615) for 30 minutes at 4 °C. After 30 minutes the beads were washed with 200 µl of Tris buffer (50mM Tris, 150 mM NaCl pH 7.4 +3 mM CaCl₂). Then 200 µl of chilled deuterium buffer (89.6% deuterium) was added and the complex was incubated for 25 minutes at 4 °C. After 25 minutes, the labeling reaction was quenched with 125 µl of chilled quench buffer on ice for 2.5 minutes. After spinning the sample for 30 seconds in a centrifuge, the quenched solution was injected onto the LC-MS system for automated pepsin digestion and peptide analysis. Similar experiments were also performed on a hCDH6(267-615) construct that contains only EC 3 through 5. In these experiments the buffer was 25 mM HEPES pH = 7.4, 150 mM NaCl, 3 mM CaCl₂ and a deuterium version of the buffer containing 94.2% deuterium.

[00393] All measurements were carried out using a minimum of three analytical triplicates. All deuterium exchange experiments were quenched using 0.5 M TCEP and 3 M urea (pH = 2.5). After quenching, the exchanged antigen was subjected to on-line pepsin digestion using a Poroszyme® Immobilized Pepsin column (2.1 x 30 mm) at 12 °C followed by trapping on a Waters Vanguard® HSS T3 (Waters Corp, Milford MA) trapping column. Peptides were eluted from the trapping column and separated on a Waters CSH C18 1 x 100 mm column (maintained at 1 °C) at a flow rate of 40

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μl/min using a binary eight minute gradient of 2 to 35% B (mobile phase A was 99.9% water and 0.1% formic acid; mobile phase B was 99.9% acetonitrile and 0.1% formic acid).

[00394] For hCDH6(54-615) protein 73% of the sequence was monitored by the deuterium exchange experiments; a complete list of these peptides appears in Table 9. Figure 3 provides a summary of the on-exchange characteristics of the hCDH6 (54-615) protein. Regions that have a greater uptake in deuterium, such as EC3, are either more solvent exposed or have a weaker hydrogen bonding network than regions that exhibit less deuterium uptake, such as regions EC4 and EC5. Overall, it is observed that EC1 through EC3 domains have greater on-exchange of deuterium than the EC4 or EC5 domains. For example, the EC2 peptide 181-199 VTATDADDPTYGNSAKVVY (SEQ ID NO:336) mean deuterium uptake is 3.61 Da; in contrast the EC5 peptide 506-525 IQTLHAVDKDDPYSGHQFSF (SEQ ID NO:337) mean deuterium uptake is only 1.18 Da. Limited deuterium uptake can make the determination of protection regions, those that undergo a shift of -0.5 Da or less upon antibody binding, more challenging due to limitations in the dynamic range.

[00395] For EC1 (54-159) the only peptides that exhibits a decrease in deuterium uptake upon binding of all four antibodies is 61-70 FLLEEYTGSD (SEQ ID NO:338). In EC1 the antibodies NOV1127 and NOV0719 demonstrate significant destabilization in the structure with changes in deuterium uptake greater or equal to 0.5 Da. Binding of NOV0710 and NOV0712 does not significantly alter the deuterium exchange behavior of the antigen in the EC1 domain.

[00396] For EC2 (160-268) the region 195-202 AKVVYSIL (SEQ ID NO:339) has a borderline decrease in deuterium uptake by all antibodies. More interestingly, the region 203-221 QGQPYFSVESETGHIKTAL (SEQ ID NO:340) is destabilized substantially (i.e. undergoes an increase in deuterium uptake) by NOV0719 and slightly less by NOV0712. Neither NOV1127 nor NOV0710 destabilize this region with a change in deuterium incorporation of +0.5 Da or greater. Lastly, the region 225-237 DRENREYQVVIQ (SEQ ID NO:341) is only destabilized upon NOV0719 binding to hCDH6 antigen.

[00397] For EC3 (269-583) the region 275-302 FKTPESSPFGTPIGRKASDADVGENAE (SEQ ID NO:342) is destabilized by NOV0719. The region 303-315 IEYSITDGEGLDM (SEQ ID NO:343) exhibits a decrease in deuterium uptake by all antibodies, but the decrease is more pronounced by NOV0710 and NOV0712 relative to the others two antibodies. The region 316-330 FDVITDQETQEGIT (SEQ ID NO:344) exhibits a decrease in deuterium uptake for all antibodies, but the decrease is the least by NOV0719. In contrast, the region 331-336 VKLLLD (SEQ ID NO:345) is destabilized by NOV0719 and less so by NOV0712. The region 337-358 FEKKKVYTLKVEASNPYVEPRF (SEQ ID NO:346) exhibits a decrease in deuterium uptake with all antibodies except NOV0719; in contrast NOV0719 is the only antibody that destabilizes the region

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337-345 FEKKKVYTL (SEQ ID NO:347). Lastly, it observed that NOV0719 also destabilizes the region 370-376 VRIVVED (SEQ ID NO:348).

[00398] For EC4 (384-486) and EC5 (487-608) the deuterium on-exchange of the antigen (Figure 3) was overall relatively low making detection of decreases in deuterium uptake challenging. For EC4 the region 407-427 AQDPDAARNPVKYSVDRHTDM (SEQ ID NO:349) exhibits a borderline decrease in deuterium uptake with NOV0710. There are no other regions in EC4 that decrease in deuterium uptake upon binding with any of the four antibodies. Overall, these observations strongly suggest that EC4 is not involved in the epitope. In contrast, 459-488 IATEINNPQSSRVPLYIKVLDVNDNAPEF (SEQ ID NO:350) exhibits various degrees of destabilization with NOV0719, NOV1127, NOV0712, and NOV0710 (listed in decreasing order of destabilization).

[00399] For EC5 only the region 564-586 YLLPVVISDNDYPVQSSSTGTVTV (SEQ ID NO:351) exhibits a borderline decrease in deuterium uptake when hCDH6 complexes with either NOV0710 or NOV0712. It should be noted that of the five extracellular domains that only EC5 does not exhibit any regions of destabilization when hCDH6 interacts with any of the four studied antibodies.

[00400] HDx experiments were also performed on hCDH6 construct consisting of only EC3-EC5, hCDH6(267-615). In these experiments the antibodies NOV0710 and NOV0712 were studied. This construct is same construct is used in crystallography experiments. The sequence coverage in these experiments was 86%. Table 10 provides a comprehensive list of all the peptides.

[00401] With this construct, in EC3 a decrease in deuterium uptake is observed in the region 295-315 ADVGENAEIEYSITDGEGLDM (SEQ ID NO:352) for both NOV0710 and NOV0712; this is very similar behavior to the full-length antigen where the region 303-315 IEYSITDGEGLDM (SEQ ID NO:343) exhibits a decrease in deuterium uptake by all antibodies, but more so by NOV0712 and NOV0710. Interestingly, there are regions in EC3 that exhibit differential behavior between the two constructs. For example, in the hCDH6(54-615) NOV0719 caused significant destabilization in many regions while the other antibodies did not. In contrast, with the hCDH6(267-615) protein both NOV0710 and NOV0712 also exhibit significant destabilization in regions that are very similar to the regions observed to be destabilized by NOV0719 in the full length hCDH6(54-615) protein.

[00402] In EC4 a similar trend is observed for destabilization. In the hCDH6(54-615) NOV0719 caused the most significant destabilization on the C-terminal side of this domain and NOV0710 and NOV0712 had little effect. In contrast, when using the hCDH6(267-615) construct both NOV0710 and NOV0712 significantly destabilize the C-terminal side of EC4. The origins for the differences in destabilization behavior are not well understood. It is important to note that in the EC4

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domain neither construct had substantial decrease in deuterium uptake with NOV0710 or NOV0712 suggesting that this region is not involved in binding NOV0710 or NOV0712.

[00403] In EC5 we observe the most consistent deuterium exchange behavior between the hCDH6 (54-615) and hCDH6 (267-615) constructs. Neither constructs exhibited significant destabilization with NOV0712 or NOV0710. Moreover, the slight and often insignificant decrease in deuterium uptake (not less than -0.5 Da) with the hCD6 (54-615) becomes more pronounced in hCDH6 (267-615). In the EC5 of hCDH6 (267-615) regions that exhibit the most significant decrease in deuterium uptake upon binding either NOV0710 or NOV0712 include the regions 492-505 YETFVCEKAKADQL (SEQ ID NO:353), 551-563 TRKNGYNRHEMST (SEQ ID NO:354), and 572-586 DNDYPVQSSTGTVT (SEQ ID NO:355). The substantial protection of 572-586 DNDYPVQSSTGTVT (SEQ ID NO:355) is in excellent agreement with the epitope data from X-ray crystallography for both NOV0710 and NOV0712. The amino acids N573, D574, and Y575 exhibit a large accessible surface area and upon formation of a complex with either antibody all three are substantially buried. V577 is also buried in both structures indicating further agreement. Crystallography data does not indicate that 492-505 YETFVCEKAKADQL (SEQ ID NO:353) is buried so this protection appears to be allosteric in nature, but the protection of 551-563 TRKNGYNRHEMST (SEQ ID NO:354) appears to be of significance especially in the NOV0712 complex where R552 is substantially buried.

[00404] Lastly, it is important to note that there are other regions in the EC5 domain that exhibit significant (-0.5 Da or less) decrease in deuterium uptake upon complex formation with either of the two antibodies. For NOV0710 these regions include 510-525 HAVDKDDPYSGHQFSF (SEQ ID NO:356), 542-550 NKDNTAGIL (SEQ ID NO:357), and 591-604 CDHHGNMQSCHAEA (SEQ ID NO:358). For NOV0712 these regions include 512-522 VDKDDPYSGHQ (SEQ ID NO:359), 542-550 NKDNTAGIL (SEQ ID NO:357), and 591-604 CDHHGNMQSCHAEA (SEQ ID NO:358).

[00405] Overall, the HDx data suggest that binding of CDH6 by the identified CDH6-binding antibodies results in several regions of the CDH6 protein becoming more prone to deuterium exchange, likely reflecting conformational changes induced by the binding of the CDH6 antibodies. The regions identified as protected from deuterium exchange in the presence of CDH6 antibodies are consistent with the x-ray crystallography data for NOV0710 and NOV0712 and form part of the epitope of these antibodies.

Table 2

Effect of NOV1127, NOV0719, NOV0710, and NOV0712 on hCDH6(54-615) containing cadherin 1-5 domains.

Peptide	Sequence	Change in Deuterium
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Name		Incorporation (Daltons)			
		NOV 1127	NOV 0719	NOV 0710	NOV 0712
61-70	FLLEEYTGSD (SEQ ID NO:338)	-1.1	-1.2	-1.1	-1.3
63-70	LEEYTGSD (SEQ ID NO:360)	-0.1	-0.3	-0.4	-0.2
71-77	YQYVGKL (SEQ ID NO:361)	0.2	0.2	-0.1	0.1
71-88	YQYVGKLHSDQDRGDGSL (SEQ ID NO:362)	0.2	0.2	-0.1	0.1
71-100	YQYVGKLHSDQDRGDGSLKYILSGDGAGDL (SEQ ID NO:363)	1.0	0.9	-0.1	0.2
90-100	YILSGDGAGDL (SEQ ID NO:364)	0	0	0	0.1
101-112	FIINENTGDIQA (SEQ ID NO:365)	0.4	0.4	0.1	0.3
101-124	FIINENTGDIQATKRLDREEKPVY (SEQ ID NO:366)	0.4	0.6	-0.3	0
101-126	FIINENTGDIQATKRLDREEKPVYIL (SEQ ID NO:367)	0.6	0.7	-0.2	0
101-128	FIINENTGDIQATKRLDREEKPVYILRA (SEQ ID NO:368)	1.1	1.1	-0.2	0.2
102-124	INENTGDIQATKRLDREEKPVY (SEQ ID NO:369)	0.4	0.5	-0.3	0.1
106-124	NTGDIQATKRLDREEKPVY (SEQ ID NO:370)	0.1	0.3	-0.4	0
106-126	NTGDIQATKRLDREEKPVYIL (SEQ ID NO:371)	0.6	0.6	-0.2	0
113-124	TKRLDREEKPVY (SEQ ID NO:372)	0.1	0.1	-0.2	-0.1

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125-144	ILRAQAINRRRTGRPVEPESE (SEQ ID NO:373)	0.3	0.4	-0.2	0.1
125-145	ILRAQAINRRRTGRPVEPESEF (SEQ ID NO:374)	0.7	0.9	0	0.3
127-144	RAQAINRRRTGRPVEPESE (SEQ ID NO:375)	0.3	0.4	-0.3	-0.1
127-145	RAQAINRRRTGRPVEPESEF (SEQ ID NO:376)	0.3	0.4	0	0.2
129-144	QAINRRRTGRPVEPESE (SEQ ID NO:377)	0.1	0.2	-0.3	-0.2
129-145	QAINRRRTGRPVEPESEF (SEQ ID NO:378)	0.2	0.3	-0.1	0.1
145-159	FIKIHNDINDNEPIF (SEQ ID NO:379)	0.9	0.9	0.4	0.5
145-162	FIKIHNDINDNEPIFTKE (SEQ ID NO:380)	0.7	0.7	0	0.2
146-159	HKIHNDINDNEPIF (SEQ ID NO:381)	0.5	0.6	0.2	0.3
146-160	HKIHNDINDNEPIFT (SEQ ID NO:382)	0.4	0.1	0.1	0.2
146-162	HKIHNDINDNEPIFTK (SEQ ID NO:383)	0.3	0.3	0	0.1
160-176	TKEVYTATVPMSDVGTF (SEQ ID NO:384)	-0.4	0.5	-0.3	-0.2
160-177	TKEVYTATVPMSDVGTF (SEQ ID NO:385)	-0.5	0.3	-0.6	-0.4
163-171	VYTATVPMSDVGTF (SEQ ID NO:386)	-0.1	0.6	0.2	0.2
177-184	FVVQVTAT (SEQ ID NO:387)	0.4	0.8	0.2	0.6
178-184	VVQVTAT (SEQ ID NO:388)	0.3	0.5	0.2	0.3
178-194	VVQVTATDADDPTYGNS (SEQ ID NO:389)	-0.1	0	-0.2	-0.1
178-199	VVQVTATDADDPTYGNSAKVVY (SEQ ID NO:390)	-0.6	-0.1	-0.8	-0.5

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181-199	VTATDADDPTYGNSAKVVY (SEQ ID NO:336)	-0.7	-0.3	-0.9	-0.6
185-199	DADDPTYGNSAKVVY (SEQ ID NO:391)	-0.3	-0.5	-0.4	-0.4
200-208	SILQQQPYF (SEQ ID NO:392)	-0.3	-0.1	-0.3	-0.3
203-211	QQQPYPFSVE (SEQ ID NO:393)	-0.1	0.5	0	0.1
203-221	QQQPYPFSVESETGHIKTAL (SEQ ID NO:340)	0.1	2.6	0.4	1.1
209-211	SVESETGHIKTAL (SEQ ID NO:394)	-0.1	2.1	0.3	0.8
212-221	SETGHIKTAL (SEQ ID NO:395)	0	1.9	0.4	0.8
214-222	TGHIKTALL (SEQ ID NO:396)	0.1	0.8	-0.1	0
222-232	LNMDRENREQY (SEQ ID NO:397)	-0.1	0	-0.1	-0.1
222-233	LNMDRENREQYQ (SEQ ID NO:398)	-0.2	0.1	-0.2	-0.1
225-233	DRENREQYQ (SEQ ID NO:399)	-0.4	-0.3	-0.4	-0.4
225-237	DRENREQYQVVQ (SEQ ID NO:341)	-0.4	1.1	-0.1	0.2
275-294	FKTPESP PGTPIGRIKASD (SEQ ID NO:400)	0.1	1.0	-0.1	0.2
275-300	FKTPESP PGTPIGRIKASDADVGEN (SEQ ID NO:401)	0.1	1.3	0	0.3
275-302	FKTPESP PGTPIGRIKASDADVGENAE (SEQ ID NO:342)	0.1	1.4	0	0.3
303-315	IEYSITDGEGLDM (SEQ ID NO:343)	-0.9	-1.1	-1.3	-1.3
303-315	YSITDGEGLDM (SEQ ID NO:402)	-0.6	-0.7	-0.8	-0.8

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306-315	SITDGEGLDM (SEQ ID NO:403)	-0.2	-0.1	-0.2	-0.1
316-326	FDVITDQETQE (SEQ ID NO:404)	-1.0	-0.6	-0.9	-0.8
316-330	FDVITDQETQEGHIT (SEQ ID NO:344)	-1.2	-0.7	-1.4	-1.5
316-336	FDVITDQETQEGHITVKLLD (SEQ ID NO:405)	-0.6	2.2	-0.8	-0.2
316-337	FDVITDQETQEGHITVKLLDF (SEQ ID NO:406)	-0.6	2.3	-0.8	-0.2
316-345	FDVITDQETQEGHITVKLLDFEKKKVYTL (SEQ ID NO:407)	-0.2	3.3	-1.1	-0.5
317-336	DVITDQETQEGHITVKLLD (SEQ ID NO:408)	-0.2	2.2	-0.1	0.6
317-337	DVITDQETQEGHITVKLLDF (SEQ ID NO:409)	-0.2	2.3	-0.1	0.6
318-337	VITDQETQEGHITVKLLDF (SEQ ID NO:410)	-0.2	2.3	-0.3	0.3
327-336	GHITVKLLD (SEQ ID NO:411)	0.1	0.9	-0.1	0.1
327-337	GHITVKLLDF (SEQ ID NO:412)	0.3	1.1	-0.1	0.1
331-358	VKKLLDFEKKKVYTLKVEASNPYVEPRF (SEQ ID NO:413)	-0.4	1.3	-1.0	-0.7
337-358	FEKKKVYTLKVEASNPYVEPRF (SEQ ID NO:346)	-0.9	0.5	-1.1	-0.9
338-345	EKKKVYTL (SEQ ID NO:414)	-0.1	-0.6	-0.2	-0.2
346-358	KVEASNPYVEPRF (SEQ ID NO:415)	-0.7	-0.4	-0.5	-0.4
370-376	VRIVVED (SEQ ID NO:348)	0.3	1.6	0	0.2

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370-386	VRIVVEDVDEPPVFSKL (SEQ ID NO:416)	0.3	1.6	0.1	0.4
376-383	DVDEPPVF (SEQ ID NO:417)	0.2	0.3	0.1	0.1
376-386	DVDEPPVFSKL (SEQ ID NO:418)	-0.1	0	-0.2	-0.2
377-383	VDEPPVF (SEQ ID NO:419)	0.3	0.6	0	0.1
377-386	VDEPPVFSKL (SEQ ID NO:420)	-0.2	-0.1	-0.4	-0.3
407-427	AQDPDAARNPVKYSVDRHTDM (SEQ ID NO:419)	-0.3	-0.3	-0.6	-0.4
413-427	ARNPVKYSVDRHTDM (SEQ ID NO:421)	-0.2	-0.2	-0.4	-0.4
420-427	SVDRHTDM (SEQ ID NO:422)	-0.1	-0.2	-0.1	-0.1
428-441	DRIFNIDSGNGSIF (SEQ ID NO:423)	1.0	0.1	-0.3	-0.3
442-451	TSKLLDRETL (SEQ ID NO:424)	0	-0.2	-0.1	-0.1
446-452	LDRETL (SEQ ID NO:425)	-0.2	-0.2	-0.2	-0.2
459-475	IATEINNPKQSSRVPLY (SEQ ID NO:426)	0.6	1.0	0.2	0.4
461-474	TEINNPKQSSRVPL (SEQ ID NO:427)	0.3	0.6	0.1	0.2
461-475	TEINNPKQSSRVPLY (SEQ ID NO:428)	0.6	0.9	0.2	0.4
462-474	EINNPKQSSRVPL (SEQ ID NO:429)	0.2	0.3	-0.1	0.1
462-475	EINNPKQSSRVPLY (SEQ ID NO:430)	0.6	0.9	0.3	0.5
462-488	EINNPKQSSRVPLYIKVLDVNDNAPEF (SEQ ID NO:431)	1.4	2.2	0.7	1.0
463-475	INNPKQSSRVPLY (SEQ ID NO:432)	0.6	0.8	0.2	0.4

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475-488	YIKVLDVNDNAPEF (SEQ ID NO:433)	0.5	0.8	0.3	0.4
476-488	IKVLDVNDNAPEF (SEQ ID NO:434)	0.5	0.7	0.2	0.4
496-505	VCEKAKADQL (SEQ ID NO:435)	-0.4	-0.4	-0.6	-0.6
506-522	IQTLHAVDKDDPYSGHQ (SEQ ID NO:436)	0.1	0	-0.1	0
506-525	IQTLHAVDKDDPYSGHQFSF (SEQ ID NO:337)	0.2	0.2	-0.1	-0.1
510-522	HAVDKDDPYSGHQ (SEQ ID NO:437)	-0.2	-0.4	-0.3	-0.3
510-524	HAVDKDDPYSGHQFS (SEQ ID NO:438)	-0.3	-0.5	-0.3	-0.3
510-525	HAVDKDDPYSGHQFSF (SEQ ID NO:356)	-0.2	-0.2	-0.3	-0.3
512-522	VDKDDPYSGHQ (SEQ ID NO:359)	-0.2	-0.4	-0.3	-0.4
512-525	VDKDDPYSGHQFSF (SEQ ID NO:439)	-0.1	-0.1	-0.3	-0.3
551-563	TRKNGYNRHEMST (SEQ ID NO:354)	-0.2	-0.1	-0.4	-0.4
587-602	RVCACDHHGNUMQSCHA (SEQ ID NO:440)	-0.1	-0.3	-0.3	-0.4
591-602	CDHHGNUMQSCHA (SEQ ID NO:441)	-0.2	-0.6	-0.4	-0.6

Table 10*Effect of NOV0710 and NOV0712 on hCDH6 (267-615) containing cadherin 3-5 domains.*

Peptide Name	Sequence	Change in Deuterium Incorporation (Daltons)	
		NOV0710	NOV0712

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274-283	QFKTPRESSPP (SEQ ID NO:442)	-0.4	-0.3
275-294	FKTPRESSPPGTPIGRIKASD (SEQ ID NO:443)	0.3	0.3
275-294	FKTPRESSPPGTPIGRIKASDA (SEQ ID NO:444)	0.3	0.4
282-294	PPGTPIGRIKASD (SEQ ID NO:445)	0.2	0.1
295-302	ADVGENAE (SEQ ID NO:446)	-0.9	-0.9
303-312	IEYSITDGEG (SEQ ID NO:447)	-0.7	-0.6
303-315	IEYSITDGEGLDM (SEQ ID NO:448)	-1.1	-1.1
306-312	SITDGEG (SEQ ID NO:449)	-0.6	-0.5
308-315	TDGEGLDM (SEQ ID NO:450)	-0.7	-0.8
316-323	FDVITDQE (SEQ ID NO:451)	0.1	0.1
316-326	FDVITDQETQE (SEQ ID NO:452)	-0.2	-0.3
316-336	FDVITDQETQEGITVKKLLD (SEQ ID NO:453)	2.2	2.0
316-337	FDVITDQETQEGITVKKLLDF (SEQ ID NO:454)	2.5	2.2
317-330	DVITDQETQEGIT (SEQ ID NO:455)	-0.8	-0.8
317-336	DVITDQETQEGITVKKLLD (SEQ ID NO:456)	2.0	1.8
318-336	VITDQETQEGITVKKLLD (SEQ ID NO:457)	2.0	1.8

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320-326	TDQETQE (SEQ ID NO:457)	-0.6	-0.6
321-336	DQETQEGITVKLLD (SEQ ID NO:458)	2.3	2.1
321-337	DQETQEGITVKLLDF (SEQ ID NO:459)	2.5	2.3
323-336	ETQEGITVKLLD (SEQ ID NO:460)	2.1	1.9
324-336	TQEGITVKLLD (SEQ ID NO:461)	1.8	1.8
327-336	GITVKLLD (SEQ ID NO:462)	1.3	1.2
327-337	GITVKLLDF (SEQ ID NO:463)	1.5	1.4
328-337	ITVKLLDF (SEQ ID NO:464)	1.4	1.3
329-336	ITVKLLD (SEQ ID NO:465)	1.1	1.0
329-337	ITVKLLDF (SEQ ID NO:466)	1.3	1.2
330-336	TVKLLD (SEQ ID NO:467)	1.1	0.9
330-337	TVKLLDF (SEQ ID NO:468)	1.3	1.0
331-337	VKLLDF (SEQ ID NO:469)	0.5	0.5
337-344	FEKKKVYT (SEQ ID NO:470)	-0.2	-0.1
337-345	FEKKKVYTL (SEQ ID NO:471)	0.5	0.6
338-345	EKKKVYTL (SEQ ID NO:472)	0.3	0.3
346-358	KVEASNPYVEPRF (SEQ ID NO:473)	-0.7	-0.4
348-358	EASNPYVEPRF (SEQ ID NO:474)	-0.9	-0.6

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359-369	LYLGPFKDSAT (SEQ ID NO:474)	-0.5	-0.3
359-370	LYLGPFKDSATV (SEQ ID NO:475)	-0.2	-0.1
361-369	LGPFKDSAT (SEQ ID NO:476)	-0.4	-0.2
370-383	VRIVVEDVDEPPVF (SEQ ID NO:477)	1.8	1.7
370-386	VRIVVEDVDEPPVFSKL (SEQ ID NO:478)	2.0	1.9
373-383	VVEDVDEPPVF (SEQ ID NO:479)	0.5	0.5
376-383	DVDEPPVF (SEQ ID NO:480)	0.4	0.4
376-386	DVDEPPVFSKL (SEQ ID NO:481)	-0.1	0.0
376-388	DVDEPPVFSKLAY (SEQ ID NO:482)	-0.3	-0.1
377-383	VDEPPVF (SEQ ID NO:483)	0.4	0.4
377-386	VDEPPVFSKL (SEQ ID NO:484)	-0.2	-0.1
407-419	AQDPDAARNPVKY (SEQ ID NO:485)	-0.1	0.1
407-427	AQDPDAARNPVKYSVDRHTDM (SEQ ID NO:486)	-0.2	-0.1
413-427	ARNPVKYSVDRHTDM (SEQ ID NO:487)	0.1	0
421-427	VDRHTDM (SEQ ID NO:488)	-0.2	-0.3
428-441	DRIFNIDSGNGSIF (SEQ ID NO:489)	0.1	0.2
442-451	TSKLLDRETL (SEQ ID NO:490)	0	0
446-452	LDRETL (SEQ ID NO:491)	-0.3	-0.1

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459-474	IATEINNPQSSRVPL (SEQ ID NO:491)	1.0	1.1
459-475	IATEINNPQSSRVPLY (SEQ ID NO:492)	1.2	1.4
461-475	TEINNPQSSRVPLY (SEQ ID NO:493)	1.0	1.0
461-478	TEINNPQSSRVPLYKV (SEQ ID NO:494)	2.5	2.6
462-474	EINNPQSSRVPL (SEQ ID NO:495)	0.7	0.8
462-475	EINNPQSSRVPLY (SEQ ID NO:496)	1.1	1.2
463-475	INNPQSSRVPLY (SEQ ID NO:497)	1.2	1.2
464-474	NNPKQSSRVPL (SEQ ID NO:498)	1.7	1.7
464-476	NNPKQSSRVPLYI (SEQ ID NO:499)	1.2	1.2
475-488	YIKVLDVNDNAPEF (SEQ ID NO:500)	1.1	1.3
476-483	IKVLDVND (SEQ ID NO:501)	1.1	1.1
476-488	IKVLDVNDNAPEF (SEQ ID NO:502)	0.8	1.0
477-488	KVLDVNDNAPEF (SEQ ID NO:503)	0.4	0.4
479-488	LDVNDNAPEF (SEQ ID NO:504)	-0.1	0.1
492-505	YETFVCEKAKADQL (SEQ ID NO:505)	-1.1	-1.1
495-505	FVCEKAKADQL (SEQ ID NO:506)	-0.7	-0.6
496-505	VCEKAKADQL (SEQ ID NO:507)	-0.8	-0.7
499-505	KAKADQL (SEQ ID NO:508)	-0.7	-0.7

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506-513	IQTLHAVD (SEQ ID NO:508)	0.3	0.4
506-522	IQTLHAVDKDDPYSGHQ (SEQ ID NO:509)	0	0.1
506-524	IQTLHAVDKDDPYSGHQFS (SEQ ID NO:510)	0.1	0.1
506-525	IQTLHAVDKDDPYSGHQFSF (SEQ ID NO:511)	-0.1	0
510-522	HAVDKDDPYSGHQ (SEQ ID NO:512)	-0.4	-0.3
510-524	HAVDKDDPYSGHQFS (SEQ ID NO:513)	-0.3	-0.2
510-525	HAVDKDDPYSGHQFSF (SEQ ID NO:516)	-0.5	-0.4
512-522	VDKDDPYSGHQ (SEQ ID NO:519)	-0.6	-0.6
512-525	VDKDDPYSGHQFSF (SEQ ID NO:514)	-0.5	-0.4
542-550	NKDNTAGIL (SEQ ID NO:357)	-0.5	-0.6
549-563	ILTRKNGYNRHEMST (SEQ ID NO:515)	-0.5	-0.4
551-563	TRKNGYNRHEMST (SEQ ID NO:354)	-0.6	-0.5
552-563	RKNGYNRHEMST (SEQ ID NO:516)	-0.7	-0.8
564-571	YLLPVVIS (SEQ ID NO:517)	-0.1	0.1
564-575	YLLPVVISDNDY (SEQ ID NO:518)	-0.5	-0.4
564-578	YLLPVVISDNDYPVQ (SEQ ID NO:519)	-1.0	-1.0
567-586	PVVISDNDYPVQSSTGTVTV (SEQ ID NO:520)	-1.5	-0.8
587-600	RVCACDHHGNMQSC (SEQ ID NO:521)	-0.5	-0.5

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587-602	RVCACDHHGNMQSCHA (SEQ ID NO:522)	-0.3	-0.3
591-600	CDHHGNMQSC (SEQ ID NO:523)	-0.7	-0.7
591-602	CDHHGNMQSCHA (SEQ ID NO:524)	-0.4	-0.4
591-604	CDHHGNMQSCHAEA (SEQ ID NO:525)	-0.6	-0.8
592-602	DHHGNMQSCHA (SEQ ID NO:526)	-0.6	-0.6
605-618	LIHPTGLSTGAGSE (SEQ ID NO:527)	-0.5	-0.3
619-625	FRHDSGL (SEQ ID NO:528)	-0.3	-0.2
619-627	FRHDSGLND (SEQ ID NO:529)	-0.4	-0.4
630-637	EAQKIEWH (SEQ ID NO:530)	-0.6	-0.5
630-638	EAQKIEWHE (SEQ ID NO:531)	-0.8	-0.7

Example 7: Epitope mapping by x-ray crystallography

[00406] The crystal structures of a human CDH6 fragment (extracellular domain 5, or EC5, SEQ ID NO: 443, Table 11) bound to Fab fragment of NOV0712 or NOV0710 (SEQ ID NO: 444-447, Table 12 and 13) were determined. As detailed below, CDH6 EC5 was co-expressed with NOV0712 or NOV0710 Fab in mammalian cells to produce a purified complex. Protein crystallography was then employed to generate atomic resolution data for CDH6 EC5 bound to NOV0712 or NOV0710 Fab and define the epitopes.

Protein production for epitope mapping

[00407] The sequences of CDH6 EC5, NOV0712 Fab and NOV0710 Fab produced for crystallography are shown in Table 9 above. The CDH6 EC5 construct comprises residues 490 to 608 shown as underlined in the context of human CDH6 (UniProt identifier P55285, SEQ ID NO: 533, Table 11), and shown below in Table 11 as SEQ ID NO: 534. The N-terminal signal sequence from mouse IgG kappa light chain is used for secreted expression of CDH6 EC5 and is cleaved during expression, leaving intact N-terminus of CDH6 EC5. The C-terminus of CDH6 EC5 is fused with a

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purification tag derived from β amyloid (APP tag, amino acids: EFRHDS (SEQ ID NO: 531)), preceded by a PreScission® protease (GE Healthcare, Piscataway, NJ) recognition site (amino acids: LEVLFQGP (SEQ ID NO: 532)) to facilitate cleavage and removal of tag after purification. For NOV0712 and NOV0710 Fab, the sequences of heavy and light chains are shown (SEQ ID NO: 535-538).

Table 11*Proteins used for structural biology studies*

Construct	Amino acid sequence in one letter code	SEQ ID NO
Human CDH6 (P55285)	MRTYRYFLLLFVVGQPYPTLSTPLSKRTSGFPARKRALEL SGNSKNELNRSKRSMWNQFFLLEEYTGSDYQYVGKLHS DQDRCDGSLKYILSGDGAGDLFIINENTGDIQATKRLDREE KPVYILRAQAINRRTGRPVEPESEFIKIHINDNEPIFTKEV YTATVPMSDVGTFVQVTATDADDPTYGNSAKVVYSIL QQQPYFSVESETGHKTALLNMDRENREQYQVVIQAKDMG GQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPP GTPIGRIKASDADVGNAEIEYSITDGEGLDMFDVITDQET QEGHITVKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFK DSATVRIVVEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPFVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD RETL WHNITVIATEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETFVCEKAKADQLIQLHAVDKDDPYSGHQFSFSLA PEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPV VISDNDYPVQSSGTGTVTVRVCACDHGNGMQSCHAEALHP TGLSTGALVAILLCIVILLVTVVLFAALRRQRKKEPLHSKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATVAYEGTGSVADSLSSLESVTTDADQDYDYLSDWG PRFKKLADMYGGVDSKDS	(SEQ ID NO: 533)
CDH6 EC5 construct	EFYETFVCEKAKADQLIQLHAVDKDDPYSGHQFSFSLAP EAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPVV ISDNDYPVQSSGTGTVTVRVCACDHGNGMQSCHAEALHP	(SEQ ID NO: 534)
NOV0712 Fab heavy chain	QVQLLESQGGGLVQPGGSLRLSCAASGFTFSHGMHWVRQ APGKGLEWVSVISGSGSNTGYADSVKGRFTISRDNKNTL YLQMNSLRAEDTAVYYCARQWGSYAFDSWGQGLVTVS	(SEQ ID NO: 535)

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	SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKRVEPKSCDKTH	
NOV0712 Fab light chain	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQOKPG KAPKLLIYAVSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQSGTFPPTTFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESV TEQDSKDSYSLSSITLTLKADYEEKHKVYACEVTHQGLSS PVTKSFNRGEC	(SEQ ID NO:536)
NOV0710 Fab heavy chain	QVQLLESQGGGLVQPGGSLRLSCAASGFTTSSYAMSWVRQ APGKGLEWVS VIRSSGSSTYYADSVKGRFTISRDN SKNTLY LQMNSLRAEDTAVYYCARGGGYFDYWQQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTH	(SEQ ID NO:537)
NOV0710 Fab light chain	DIQMTQSPSSLSASVGDRVTITCRASQSI SLWLNWYQOKPG KAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDF ATYYCQQVYTSPTYTFGQGTKVEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESV TEQDSKDSYSLSSITLTLKADYEEKHKVYACEVTHQGLSS PVTKSFNRGEC	(SEQ ID NO:538)
CDH6-D574A	MRTYRYFLLLLFWVQQPYPTLSTPLSKRTSGFPAKKRALEL SGNSKNELNRSKRSMWNQFFLLEEYTGSDYQYVGKLHS DQDRGDGSLKYILSGDGAGDLFIINENTGDIQATKRLDREE KPVYILRAQAINRRRTGRPVEPESEFIHKIHDINDNEPIFTKEV YTATVPMSDVGTFVQVTATDADDPTYGNSAKVVYSIL QQQPYFSVESETGHIKTALLNMDRENREQYQVVIQAKDMG GQMGGLSGTTTNITLTDVNDNPPRFPPQSTYQFKTPESPP GTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQET QEGHITVKLLDFEKKKVYTLKVEASNYPYVEPRFLYLGPFFK DSATVRIVVEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD RETL LWHNITVIA TEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLA PEAASGSNFTIQDNKDN TAGILTRKNGYNRHEMSTYLLPV VISDNDYPVQSSSTGTVTVRVCACDHHGNMQSCHAEALHP	(SEQ ID NO:539)

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	TGLSTGALVAILLCIVILLVTVVLFAALRRQRKKEPLHSKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATYAYEGTGSVADSLSSLESVTTDADQDYDYLSDWG PRFKKLADMYGGVDSKDSD	
CDH6-Y575A	MRTYRYFLLLFVVGQPYPTLSTPLSKRTSGFPAKKRALEL SGNSKNELNRSKRSWMWNQFFLLEEYTGSDYQYVGKLHS DQDRGDGSLKYILSGDGAGDLFIHENTGDIQATKRLDREE KPVYILRAQAINRRRTGRPVEPESEFIKIHNDINDNEPIFTKEV YTATVPMSDVGTFVQVTATDADDDPTYGNSAKVVYSIL QQQPYFSVESETGHIKTALLNMDRENREQYQVVIQAKDMG GQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPP GTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQET QEGHITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFFK DSATVRIVVEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD RETLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETVCEKAKADQLIQLTHAVDKDDPYSGHQFSFSLA PEAASCSNFTIQDNKDNNTAGILTRKNOYNRHEMSTYLLPV VISDNDYPVQSSSTGTVTVRVCACDHHGNMQSCHAEALHP TGLSTGALVAILLCIVILLVTVVLFAALRRQRKKEPLHSKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATYAYEGTGSVADSLSSLESVTTDADQDYDYLSDWG PRFKKLADMYGGVDSKDSD	(SEQ ID NO: 540)
CDH6-N573A	MRTYRYFLLLFVVGQPYPTLSTPLSKRTSGFPAKKRALEL SGNSKNELNRSKRSWMWNQFFLLEEYTGSDYQYVGKLHS DQDRGDGSLKYILSGDGAGDLFIHENTGDIQATKRLDREE KPVYILRAQAINRRRTGRPVEPESEFIKIHNDINDNEPIFTKEV YTATVPMSDVGTFVQVTATDADDDPTYGNSAKVVYSIL QQQPYFSVESETGHIKTALLNMDRENREQYQVVIQAKDMG GQMGGLSGTTTVNITLTDVNDNPPRFPQSTYQFKTPESSPP GTPIGRIKASDADVGENAEIEYSITDGEGLDMFDVITDQET QEGHITVKKLLDFEKKKVYTLKVEASNPYVEPRFLYLGPFFK DSATVRIVVEDVDEPPVFSKLAYILQIREDAQINTTIGSVTA QDPDAARNPVKYSVDRHTDMDRIFNIDSGNGSIFTSKLLD	(SEQ ID NO: 541)

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RETLWHNITVIATEINNPKQSSRVPLYIKVLDVNDNAPEF AEFYETVCEKAKADQLIQTLHAVDKDDPYSGHQFSFSLA PEAASGSNFTIQDNKDNTAGILTRKNGYNRHEMSTYLLPV VISDNDYPVQSSGTGTVTVRVACDHHGNGMQSCHAEALHP TGLSTGALVAILLCIVILLVTVVLFAALRRQRKKEPLHSKE DIRDNIVSYNDEGGGEEDTQAFDIGTLRNPEAIEDNKLRRD IVPEALFLPRRTPTARDNTDVRDFINQRLKENDTDPTAPPY DSLATYAYEGTGSVADSLSSLESVTTDADQDYDYLSDWG PRFKKLADMYGGVDSKDSD	
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[00408] CDH6 EC5 was co-expressed with NOV0712 or NOV0710 Fab in Expi293® cells to produce complex for crystallography. In detail, 0.5 mg of plasmid encoding CDH6 EC5 was mixed with 0.5 mg of NOV0712 or NOV0710 Fab plasmid, diluted into 50ml of OptiMEM 1 medium (Life Technologies, Grand Island, NY), and incubated with 2.5mg of PEI (Polysciences, Warrington, PA) in 50ml of the same medium for 30 minutes. The mixture was then added into 1L of Expi293® cells growing in suspension in Expi293® Expression medium (Life Technologies Grand Island, NY) at 1 million cells/ml at 37 °C with 8% of CO₂ for transfection. After 72 hours, the medium which contains CDH6 EC5-Fab complex was harvested by centrifugation, and supplemented with CaCl₂ to 3mM. 10ml of Sepharose® 4B beads (GE Healthcare Pittsburgh, PA) conjugated with an anti-APP tag IgG (generated in-house) were then added into the medium and kept stirring at 4 °C overnight. The next day the beads were packed into a gravity column and washed with 25mM Hepes pH 7.4 + 150mM NaCl (HBS), and 3mM CaCl₂. The target complex was eluted with 3 column volumes (CV) of 100 mM glycine pH 2.5, 150mM NaCl, 3mM CaCl₂, into 1/10 (v/v) of 1 M Tris pH 8.5. The complex was incubated with 1/100 (w/w) GST-tagged PreScission® protease (GE Healthcare, Pittsburgh, PA) at 4 °C overnight to cleave APP tag. The next day, PreScission® protease was removed by passing the mixture through a 1ml GSTrap® HP column (GE Healthcare, Pittsburgh, PA). The flow-through was then incubated with 1/10 (w/w) of PNGaseF (purified in house) at 37 °C overnight to remove N-linked glycosylation. After deglycosylation, the mixture was concentrated and loaded onto HiLoad 16/600 Superdex® 200 pg (GE Healthcare, Pittsburgh, PA) equilibrated in HBS plus 3 mM CaCl₂. Peak fractions containing purified CDH6-Fab complex were analyzed by SDS-PAGE and LCMS, pooled and concentrated for crystallization.

Crystallization and structure determination

[00409] CDH6 EC5/NOV0712 and CDH6 EC5/NOV0710 complexes were concentrated to 9.3 mg/ml and 14.7 mg/ml, respectively, centrifuged at 20,000 g for 5 minutes, and screened for crystallization. Crystals for data collection were grown by hanging drop vapor diffusion at 20 °C.

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Crystals of CDH6 EC5/NOV0712 complex were grown by mixing 1.2 μ l of the complex with 1 μ l of reservoir solution containing 20% (v/v) PEG3350 and 0.2 M di-ammonium hydrogen citrate, and equilibrating the drop against 450 μ l of the same reservoir solution plus 50 μ l water. Crystals of CDH6 EC5/NOV0710 complex are grown by mixing 0.8 μ l of the complex with 1 μ l of reservoir solution containing 0.7 M tri-sodium citrate and 0.1 M Tris•HCl pH 8.5, and equilibrating the drop against 425 μ l of the same reservoir solution plus 75 μ l water. Before data collection, the crystals were transferred to 75% of reservoir solutions plus 25% glycerol and flash cooled in liquid nitrogen.

[00410] Diffraction data were collected at beamline 17-ID at the Advanced Photon Source (Argonne National Laboratory, USA). Data are processed and scaled using Autoproc (Global Phasing, LTD). The data of CDH6 EC5/NOV0712 was processed to 2.3 Å in space group P212121 with cell dimensions $a=78.83$ Å, $b=88.86$ Å, $c=186.73$ Å, $\alpha=90^\circ$, $\beta=90^\circ$, $\gamma=90^\circ$. The data of CDH6 EC5/NOV0710 complex was processed to 3.5 Å in space group P43212 with cell dimensions $a=101.48$ Å, $b=101.48$ Å, $c=240.96$ Å, $\alpha=90^\circ$, $\beta=90^\circ$, $\gamma=90^\circ$. The structures of the complexes were solved by molecular replacement using Phaser (McCoy et al., (2007) J. Appl. Cryst. 40:658-674) with in-house Fab structures as search models. The structure of CDH6 EC5 was built from scratch using Buccaneer (K. Cowtan (2006) Acta Cryst. D62:1002-1011) in the CCP4 program suite (Winn et al., (2011) Acta. Cryst. D67:235-242). The final models were built in COOT (Emsley & Cowtan (2004) Acta Cryst. D60:2126-2132) and refined with Buster (Global Phasing, LTD, Cambridge, UK). For the CDH6 EC5/NOV0712 complex, the Rwork and Rfree values are 21.2% and 25.2%, respectively; and root-mean-square (r.m.s) deviation values of bond lengths and bond angles are 0.010 Å and 1.24°, respectively. For the CDH6 EC5/NOV0710 complex, the Rwork and Rfree values are 18.2% and 24.6%, respectively; and r.m.s deviation values of bond lengths and bond angles are 0.010 Å and 1.28°, respectively.

[00411] Residues of CDH6 EC5 that are in contact with NOV0712 or NOV0710 Fab, the types of interactions, and the buried surface areas are all identified by PISA (Krissinel et al., (2007) J Mol Biol. 372:774-97) and listed in Tables 12 and 13. For the structures that contain more than one copy of complex in the asymmetric unit (the smallest unique unit in the crystal), only those antibody-contacting residues that are common in all copies are listed as epitope residues.

Epitopes of NOV0712 and NOV0710 on CDH6

Overall structures

[00412] There is no reported crystal structure of CDH6 EC5 at this time. The overall folding of CDH6 EC5 is similar to that of CDH2 (N-Cadherin) EC5, both composed by one three-strand β sheet stacking against another four-strand β sheet. Overlay of the two structures results in a small root-mean-square distance (RMSD) of 2.4 Å and similar orientation of the N- and C-terminus. Thus one can overlay the CDH6 EC5/Fab complex structure onto the full-length ECD structure of CDH2 based on

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the superposition of EC5 domains, in order to estimate the orientations and NOV0712 and NOV0710 Fab. As shown in Figure 4, NOV0712 and NOV0710 bind the same side of CDH6 EC5, and share a significantly overlapped epitope. NOV0712 binds slightly shifted away from the EC4-EC5 linker compared to NOV0710.

Epitope of NOV0712

[00413] The crystal structure of the CDH6 EC5/NOV0712 complex is used to identify the NOV0712 epitope on CDH6. The interaction surface on CDH6 EC5 by NOV0712 Fab is formed by several continuous and discontinuous (i.e. noncontiguous) sequences: namely residues 503, 520-527, 529, 532-534, 538-543, 550, 552, 569, and 571-577, as detailed in Table 12. These residues form the three-dimensional conformational epitope that is recognized by NOV0712 Fab (Figure 5 upper panel). This epitope defined by crystallography is in good agreement with that defined by hydrogen deuterium exchange mass spectrometry (HDX-MS), in which residues 572-586 are substantially protected by NOV0712. Interestingly, a potential Ca²⁺-binding loop of CDH6 EC5 (residues 571-579) has majority of it (8 out of 10 residues) in contact with NOV0712, especially Tyr575 and Asn573 which are almost completely buried by the antibody (Table 12 and Figure 5 lower panel). Compared with CDH2 structure, this Ca²⁺-binding loop of CDH6 EC5 is in an "out" conformation induced by NOV0712 binding (e.g. Asp572 of CDH6 is flipped out compared with its corresponding residue Asp525 in CDH2).

Table 12

NOV0712 epitope on CDH6. All residues of CDH6 that are in contact with NOV0712 in the crystal structure are identified by PISA, listed and sorted by their buried surface area by NOV0712. Types of interaction are also listed where applicable.

Residue name	Residue number	Accessible surface area (ASA, Å ²)	Buried surface area (BSA) by heavy chain (Å ²)	BSA by light chain (Å ²)	Total BSA by NOV0712 (Å ²)	BSA/ASA (%)	Type of interaction
TYR	575	205.77	131.73	47.11	178.84	86.91	H-SC ¹ , H-MC ² , Hydrophobic
ASN	573	106.72	103.67	0	103.67	97.14	H-SC, H-MC
LYS	543	147.48	65.84	37.33	103.17	69.96	Salt bridge
ARG	552	148.25	0	78.9	78.9	53.22	H-SC
ASP	574	104.57	68.99	0	68.99	65.97	H-SC, H-MC
GLN	540	99.91	0	68.29	68.29	68.35	H-SC
PRO	529	122.44	0	65.25	65.25	53.29	
ALA	532	67.01	0	64.9	64.9	96.85	H-MC
SER	533	103.84	0	59.58	59.58	57.38	H-SC

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VAL	577	97.82	53.24	0	53.24	54.43	H-MC
SER	526	52.04	0	47.12	47.12	90.55	
ASP	541	45.28	8.86	36.42	45.28	100.00	H-MC
GLN	522	100.04	45.14	0	45.14	45.12	
SER	524	43.52	26.98	16.54	43.52	100.00	
GLY	520	64.48	32.88	0	32.88	50.99	H-MC
ASP	503	113.22	0	32.06	32.06	28.32	
ILE	539	31.09	0	29.09	29.09	93.57	H-MC
HIS	521	104.3	23.91	0	23.91	22.92	
THR	538	39.69	0	23	23	57.95	
LEU	527	24.51	0	20.01	20.01	81.64	H-MC
PRO	576	111.16	17.57	0	17.57	15.81	
ASN	542	72.05	0	17.39	17.39	24.14	
PHE	525	15.47	0.95	14.52	15.47	100.00	
SER	571	16.16	6.96	7.1	14.06	87.00	
VAL	569	23.13	0	8.54	8.54	56.92	
LEU	550	23.71	0	7.9	7.9	33.32	
GLY	534	71.3	0	5.13	5.13	7.19	
PHE	523	9.81	4.5	0	4.5	45.87	
ASP	572	23.71	2.26	0	2.26	9.53	

¹Hydrogen bond by side-chain atoms; ²Hydrogen bond by main-chain atoms

Epitope of NOV0710

[00414] The crystal structure of the CDH6 EC5/NOV0710 complex is used to identify the NOV0710 epitope on CDH6. The interaction surface on CDH6 EC5 by NOV0712 Fab is formed by several continuous and discontinuous (i.e. noncontiguous) sequences: namely residues 520-527, 529, 541, 543, 569, and 571-579, as detailed in Table 13. These residues form the three-dimensional conformational epitope that is recognized by NOV0710 Fab (Figure 6 upper panel). This epitope defined by crystallography is in good agreement with that defined by hydrogen deuterium exchange mass spectrometry (HDX-MS), in which residues 572-586 are substantially protected by NOV0710. Interestingly, similar as in the CDH6 EC5/NOV0712 complex structure, all residues in the potential Ca²⁺-binding loop of CDH6 EC5 (residues 571-579) are in contact with NOV0710; among which Tyr575, Asn573 and Asp574 are almost completely buried by the antibody (Table 13 and Figure 6 lower panel). Compared with CDH2 structure, this Ca²⁺-binding loop of CDH6 EC5 is in an "out" conformation induced by NOV0710 binding (e.g. Asp572 of CDH6 is flipped out compared with its corresponding residue Asp525 in CDH2).

Table 13

NOV0710 epitope on CDH6. All residues of CDH6 that are in contact with NOV0710 in the crystal structure are identified by PISA, listed and sorted by their buried surface area by NOV0710. Types of interaction are also listed where applicable.

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Residue name	Residue number	Accessible surface area (ASA) ($\text{\AA}^2$)	Buried surface area (BSA) by heavy chain ($\text{\AA}^2$)	BSA by light chain ($\text{\AA}^2$)	Total BSA by NOV0710 ($\text{\AA}^2$)	BSA / ASA (%)	Type of interaction
TYR	573	201.38	147.28	54.1	201.38	100.00	H-MC ¹ , Hydrophobic
ASN	573	128.75	116.17	4.34	120.51	93.60	H-SC ² , H-MC
ASP	574	95.9	87.31	0	87.31	91.04	Salt bridge
LYS	543	145.5	0	78.92	78.92	54.24	H-SC
VAL	577	93.73	21.59	52.36	73.95	78.90	
PRO	529	133.4	0	72.75	72.75	54.54	
SER	526	58.42	0	46.58	46.58	79.73	
SER	524	40.31	17.45	22.86	40.31	100.00	H-SC
GLN	522	108.66	29.79	0	29.79	27.42	
SER	571	28.68	0.34	24.21	24.55	85.60	
PRO	576	83.21	24.36	0	24.36	29.28	H-MC
ASP	541	33.17	0	22.2	22.2	66.93	H-SC
GLY	520	74.53	17.18	0	17.18	23.05	
VAL	569	26.11	0	16.9	16.9	64.73	
SER	579	82.89	0	13.6	13.6	16.41	
GLN	578	116.86	13.18	0	13.18	11.28	
HIS	521	84.59	10.25	0	10.25	12.12	
LEU	527	41.01	0	10.07	10.07	24.55	
ASP	572	18.64	6.51	0	6.51	34.92	Salt bridge
PHE	525	9.03	0	5.15	5.15	57.03	
PHE	523	15.28	0.31	0	0.31	2.03	

¹Hydrogen bond by main-chain atoms; ²Hydrogen bond by side-chain atoms

Example 8: ELISA assay to validate co-crystal structure and determine binding of anti-CDH6 antibodies to wild-type and mutant CDH6 proteins.

[00415] Analysis of the CDH6 protein/NOV0710 or /NOV0712 crystal structures highlighted several amino acid residues (Asn573, Asp574 and Tyr575) with high buried surface values, suggesting they might be important for mediating the interaction of the antibodies with the CDH6 protein. We produced recombinant mutant CDH6 protein, replacing these residues by alanine (Table 11, SEQ ID NOs. 539, 540 and 541) and performed ELISA. 96-well Maxisorp plates (Nunc) were coated overnight at 4 °C with 1 µg/ml, 100µl/well of the appropriate recombinant human protein (CDH6 wt, CDH6-N573A, CDH6-D574A or CDH6-Y575A). All wells were then washed three times with PBS/ 0.1% Tween-20, blocked for one hour with PBS/ 1% BSA/ 0.1% Tween-20 and washed three times

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with PBS/ 0.1% Tween-20. A serial dilution of anti-CDH6 antibodies was added to the relevant wells (100 nM starting concentration; 5-fold serial dilution) and plates were incubated at room temperature for two hours. Plates were washed three times with PBS/ 0.1% Tween-20 prior to the addition of a goat anti-human peroxidase linked detection antibody (Pierce, #31412, Thermo, Rockford, IL), diluted 1/10000 in PBS/ 1% BSA/ 0.1% Tween-20. Plates were incubated at room temperature for one hour before washing three times with PBS/ 0.1% Tween-20. 100 µl TMB (3,3', 5,5' tetramethyl benzidine) substrate solution (BioRx) was added to all wells for 6 minutes before stopping the reaction with 50 µl 2.5% H2SO4. The extent of CDH6 antibody binding to each recombinant protein was determined by measuring the OD450 using a SpectraMax plate reader (Molecular Devices). Dose response curves were analyzed using Graphpad Prism.

[00416] As shown in Figure 7, the D574A mutation abrogated binding of both NOV0710 and NOV712, confirming this residue is an essential component of a shared epitope of the two antibodies. Interestingly, the N573A mutant retains binding to NOV712 but not NOV0710, whereas the Y575A mutant retains binding to NOV0710 but not NOV0712. Of note, neither of these mutations impacted binding of the anti-CDH6 antibody NOV0720, which binds a distinct epitope (NOV0720 is the non-germlined version of NOV1132 in Figure 2) -- indicating the mutants did not alter overall architecture of the proteins. These data further validate the co-crystal structures and confirm that the two antibodies feature overlapping, yet distinct epitopes.

Example 9: Preparation of antibody drug conjugates

Preparation of DM4 Conjugates by One-Step Process

[00417] An anti-CDH6 antibody, for example NOV7012, at a concentration of about 10.0 mg/mL, was mixed with DM4 (6.8-fold molar excess relative to the amount of antibody) and then with sulfo-SPDB (about 5.2-fold excess relative to the amount of antibody). The reaction was performed at 20° C in 20 mM EPPS [4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid] buffer (pH 8.1) containing 10% DMA for approximately 16 hours. The reaction was quenched by adding 1 M acetic acid to adjust the pH to 5.0. After pH adjustment, the reaction mixture was filtered through a multi-layer (0.45/0.22 µm) PVDF filter and purified and diafiltered into a 10 mM succinate buffer (pH 4.5) using Tangential Flow Filtration. An example of the instrument parameters for the Tangential Flow Filtration are listed in Table 14 below.

Table 14

Instrument parameters for the Tangential Flow Filtration

TFF Parameter	TFF Set Point
Bulk Concentration (Cb -- g/L)	20

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TMP (psi)	12-18
Feed Flow rate (LMH)	324
Membrane Load (g/m ²)	50-150
Diavolumes	16
Diafiltration Buffer	20 mM Succinate, pH 4.5
Temperature (°C)	room temperature (20 – 25)

[00418] Conjugates obtained from the process described above were analyzed by: UV spectroscopy for cytotoxic agent loading (Maytansinoid to Antibody Ratio, MAR); SEC-HPLC for determination of conjugate monomer; and reverse-phase HPLC or hydrophobic shielded phase (Hisep)-HPLC for free maytansinoid percentage.

Preparation of ADCs with the SPDB or sulfo-SPDB linker and DM4 cytotoxic agent

[00419] Anti-CDH6 antibodies, for example, antibody NOV0712, were combined with DM4 (6.8 and 10.2-fold molar excess) in 50 mM EPPS buffer (pH 8.1) containing ~10 % DMA at 25 °C. To this mixture was added N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB, 4.0 and 6.0-fold molar excess, respectively) such that the final antibody concentration was 4 mg/mL and the final DMA content was 10%. The reaction was allowed to proceed for ~16 hours at 25 °C in 50 EPPS buffer (pH 8.1). The conjugation reaction mixture was purified using a SEPHADEX™ G25 column equilibrated and eluted with 10 mM succinate, 250 mM glycine, 0.5% sucrose, 0.01% Tween 20, pH 5.5. Similar procedures were used to generate anti-CDH6-sulfo-SPDB-DM4 conjugates

[00420] Either of the above methods is useful in the conjugation of antibodies with SPDB-DM4 or sulfo-SPDB-DM4. Table 15 below provides an example of CDH6 ADCs.

Table 15

Properties of DM4-conjugated antibodies

Ab	MAR	Monomer %	Yield (%)	Free DM (%)
NOV0709	3.8	97	84	1.6
NOV0691	3.6	96	97	1.5
NOV0690	3.2	97	74	4.0
NOV0689	3.3	99	96	2.0
NOV0670	3.1	98	100	1.5
NOV0720	3.3	99	78	2.0
NOV0712	3.2	99	78	3.1
NOV0674	3.2	99	78	2.6
NOV0719	3.8	98	79	1.9

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Preparation of SMCC-DM1 Conjugates by in situ Process

[00421] The anti-CDH6 antibodies can also be conjugated with SMCC-DM1 using an in situ process according to the following procedures. CDH6 antibodies were conjugated to DM1 using the sulfosuccinimidy 1 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) linker. Stock solutions of DM1 and sulfo-SMCC heterobifunctional linker were prepared in DMA. Sulfo-SMCC and DM1 thiol were mixed together to react for 10 minutes at 25°C in DMA containing 40% v/v of aqueous 50 mM succinate buffer, 2 mM EDTA, pH 5.0, at the ratio of DM1 to linker of 1.3:1 mole equivalent and a final concentration of DM1 of 1.95 mM. The antibody was then reacted with an aliquot of the above in situ generated SMCC-DM1 so that the final conjugation conditions included 2.5 mg/mL of Ab in 50 mM EPPS, pH 8.0, 10% DMA (v/v) and a molar ratio of SMCC:Ab around 6.5. After approximately 18 hours at 25°C, the conjugation reaction mixture was purified using a SEPHADEX® G25 column equilibrated with 10 mM succinate, 250 mM glycine, 0.5% sucrose, 0.01% Tween 20, pH 5.5.

Example 10: Validation of anti-CDH6 antibody and ADC binding in a panel of CHO cell lines expressing CDH6 from human, cynomolgus, rat or mouse origins.

[00422] To validate unconjugated anti-CDH6 antibody and anti-CDH6-ADC binding to cells featuring expression of CDH6 from different species, FACS (Fluorescence Activated Cell sorting) analysis was performed on CHO cells engineered to express CDH6 from human, cynomolgus, rat and mouse origins. The generation of the CHO cell lines featuring CDH6 expression is described in Example 1; FACS methods are described in Example 3.

[00423] As shown in Figure 8, non-conjugated CDH6 antibody NOV0712 features comparable cellular binding to CDH6 from human, cynomolgus, rat and mouse origin. The NOV0712-sulfo-SPDB-DM4 antibody drug conjugate features a comparable binding profile to non-conjugated NOV0712. This result indicates that the addition of a linker and payload does not interfere with binding affinity or specificity of anti-CDH6 antibodies.

Example 11: Assessment of CDH6 expression and cellular binding of CDH6-targeting antibodies and ADCs in a panel of ovarian cancer cell lines.

[00424] In preparation for evaluation of cellular activity of CDH6-targeting ADCs, the CDH6 cell surface expression levels were assessed in a panel of ovarian cancer cell lines by FACS. Specifically, a cell suspension was prepared by treating cells in culture with Accutase® Cell Dissociation Reagent (Gibco, #A1110501 Grand Island, NY) according to the manufacturer's instructions followed by washing the cells in FACS buffer (RPMI/1%BSA, Gibco, Grand Island, NY). Cells were resuspended in FACS buffer at 1×10^6 cells/mL and aliquoted into a 96-well round bottom plate at 100 μ L/well (Corning #CLS3360 Tewksbury, MA). Primary antibodies (anti-CDH6-PE (R&D Systems, #FAB2715P Minneapolis, MN) and control mouse IgG1 PE conjugate (R&D Systems,

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#IC002P Minneapolis, MN) were diluted in FACS buffer at 5 µg/ml. Following three washes in 200µl cold FACS buffer, cells were analyzed on a BD FACS Canto II® (BD Biosciences, San Jose, CA). Geomean of signal per sample was determined using FlowJo® software.

[00425] OVCAR3 endogenously express high levels of CDH6. To generate an isogenic cell line with suppressed CDH6 expression, OVCAR3 were transduced with a lentiviral vector delivering a shRNA targeting CDH6 according to the manufacturer's instructions (Mission shRNA bacterial glycerol stock, #TRCN0000054117, Sigma, St. Louis, MO). OVCAR8 cells do not express CDH6 endogenously. To generate an isogenic cell line featuring CDH6 expression, OVCAR8 cells were transduced with a lentiviral construct driving expression of a human CDH6 cDNA. Specifically, the cDNA for CDH6 was purchased from Genecopoeia (#Z2028, Genecopoeia, Rockville, MA) and cloned into pLenti6.3 using Gateway® cloning according to the manufacturer's instructions (Invitrogen, Carlsbad, CA).

[00426] FACS analysis confirmed high levels of endogenous CDH6 expression in wild-type OVCAR3 and suppression of CDH6 in the OVCAR3 shRNA^{CDH6} cell line. OVCAR8 cells were confirmed to be CDH6-negative, while high levels of exogenous expression of CDH6 were observed in the OVCAR8 engineered CDH6⁺ cells (Figure 9).

[00427] FACS analysis further confirmed specific cellular binding of generated CDH6-targeting antibodies and ADCs. As shown in Figure 10, a non-conjugated CDH6 antibody (NOV0712) features CDH6-specific binding pattern comparable to that of a sulfo-SPDB-DM4 conjugate using the same antibody and the sulfo-SPDB-DM4 linker/payload. A non-binding unconjugated IgG or ADC was included as control and found not to result in cell binding as expected. Again, indicating that the addition of a linker and payload does not interfere with affinity or specificity of anti-CDH6 antibodies.

Example 12: In vitro cellular activity of CDH6-targeting ADCs

[00428] *In vitro* cellular activity of SMCC-DM1 conjugates of a selection of anti-CDH6 antibodies was determined on a panel of cell lines comprising OVCAR3 (ovarian serous carcinoma, cultured in RPMI + 20% FCS), JHOS4 (1:1 F12:DMEM+10%FBS), KNS42 (EMEM+10%FBS), NCIH661 (RPMI+10%FBS), SNU8 (RPMI+10%FBS) and OVCAR8 (RPMI+10%FBS). The OVCAR3 and NCIH661 cell lines were obtained from ATCC (#HTB-161 and #HTB-183, ATCC Manassas, VA). JHOS4 were obtained from RIKEN (#RCB1678, RIKEN Cell Bank, RIKEN, Tsukuba, Japan). KNS42 were obtained from the Health Science Research Resources Bank HSRRB (#IFO50356, HSRRB, Osaka, Japan). SNU8 were obtained from the Korean Cell Line Bank KCLB (#00008, KCLB, Seoul, Korea). OVCAR8 were obtained from the NCI/DC/CTD Tumor/Cell Line Repository (NCI, Frederick, MA).

[00429] Cells in culture were counted and diluted in medium to a concentration of 1×10^5 cells/ml. 1000 cells/well were transferred to 384-well plates (Corning Costar#3707, Corning,

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Tewksbury, MA). The ADC stock solution was prepared in 1.4ml Matrix tubes (Thermo, # 3790, Rockford, IL). A 10-point, 1:3 serial dilution was prepared in a 384-well deep-well plate (Brundtech Scientific Inc #701353, Essex, CT) and 25 μ l were transferred per assay plate (triplicates) to yield a highest starting concentration of the ADC of 33nM. For controls, wells with cells only (=100% viability control) and cells incubated with an SMCC-DM1 conjugated non-targeting antibody (to check for non-target driven activity) were prepared. Plates were incubated for 120 h at 37°C and 5% CO₂. Cellular activity of the primary antibody/Fab-DM1 complexes was determined using CellTiter-Glo® reagent (Promega #G7571, Madison, WI) according to the manufacturer's instructions. Viability was normalized to the cells only control and data were plotted using Tibco Spotfire (Tibco Software Inc, Palo Alto, CA)

[00430] A subset of CDH6-targeting ADCs is able to inhibit proliferation of CDH6-positive cells at concentrations (1.22 nM) that are inactive on cells that lack CDH6 expression (OVCAR8), indicating target-dependent *in vitro* cellular activity of the CDH6-ADC (Table 16).

Table 16

In vitro cellular activity of anti-CDH6 SMCC-DM1 conjugates. Table shows percent inhibition at 1.22nM ADC.

Antibody	OVCAR3	JHOS4	KNS42	NCH661	SNU8	OVCAR8
NOV0670	-29.97	-27.88	-18.63	5.14	-15.57	-23.77
NOV0672	-16.25	-22.96	-12.26	-6.14	-10.94	-16.16
NOV0674	-10.29	-11.94	-6.82	3.9	-1.42	-8.97
NOV0682	61.75	33.92	23.75	16.44	10.83	-2.17
NOV0685	-20.65	-14.67	-14.65	2.08	-0.1	-11.51
NOV0689	7.21	3.63	-3.11	-0.53	10.27	-7.29
NOV0690	84.8	50.97	56.81	14.55	18.39	-15.8
NOV0691	-1.5	-14.37	-9.23	-10.47	-2.56	-10.48
NOV0692	88.01	62.3	57.32	14.77	15.66	-10.83
NOV0693	85.73	58.08	15.17	25.87	35.56	-7.07
NOV0695	89	66.94	66.31	18.69	21.56	-7.04
NOV0699	n/a	n/a	n/a	n/a	n/a	n/a
NOV0705	68.57	40.35	-13.35	-3.15	6.4	-22.58
NOV0709	46.88	4.5	-4.2	5.91	-7.7	-8.34
NOV0710	90.84	69.01	56.98	38.7	42.4	6.53
NOV0712	87.62	67.56	56.82	32.46	31.35	1.68
NOV0713	-3.55	-6.22	-17.26	-9.74	7.79	-0.08
NOV0718	-3.8	-5.03	3.5	2.51	3.35	6.64
NOV0719	76.05	27.25	40.97	-9.87	3.18	-2
NOV0720	80.72	43.52	56.97	1.94	3.56	-3.9

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Example 13: In vitro cellular activity of anti-CDH6 ADCs as SMCC-DM1 and SPDB-DM4 conjugates on ovarian cancer cell lines

[00431] In vitro cellular activity of anti-CDH6 ADCs as SMCC-DM1 and SPDB-DM4 conjugates was evaluated on a panel of ovarian cancer cell lines (Figure 11 and Table 17). To assess target-dependent and specific activity of the anti-CDH6 ADCs, isogenic cell line pairs were generated for the OVCAR3 and OVCAR8 cell lines as described in Example 11.

[00432] CDH6-targeting ADCs in both SMCC-DM1 and SPDB-DM4 formats exhibited target and dose-dependent cellular activity as indicated by inhibition of proliferation in CDH6 expressing cell lines compared to the non-expressing isogenic cell line pair and isotype control ADCs (Figure 11 and Table 17). The cellular activity assay was performed and analyzed as disclosed in Example 12.

Table 17

In vitro cellular activity of anti-CDH6 ADCs as SMCC-DM1 and SPDB-DM4 conjugates on ovarian cancer cell lines.

Compound	Linker/ Payload	OVCAR3		OVCAR3 shRNA_CDH6		OVCAR8		OVCAR8 (engineered CDH6+)	
		IP [nM]	Amax (%)	IP [nM]	Amax (%)	IP [nM]	Amax (%)	IP [nM]	Amax (%)
mc-DM1	free payload	0.002	90	0.002	89	0.104	85	0.087	84
IgG	SMCC- DM1	29.993	91	18.468	92	33	n/a	33	n/a
IgG	SPDB- DM4	3.966	91	2.168	91	17.79	79	32.728	83
NOV0710	SMCC- DM1	1.034	101	18.52	93	33	5	0.102	94
NOV0710	SPDB- DM4	0.01	88	7.543	91	33	n/a	0.018	85
NOV0712	SMCC- DM1	0.065	88	11.249	94	33	n/a	0.015	95
NOV0712	SPDB- DM4	0.008	88	3.785	90	33	n/a	0.012	84

Example 14: In vitro cellular activity of CDH6-targeting antibodies or control IgG in either non-conjugated form or as sulfo-SPDB-DM4 antibody drug conjugate.

[00433] Cellular activity of the CDH6 antibody NOV0712 as either non-conjugated IgG or sulfo-SPDB-DM4 conjugate was assessed in a panel of ovarian cancer cell lines (see Example 11). A

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non-targeting isotype control antibody in either non-conjugated form or as sulfo-SPDB-DM4 conjugate was further included. As shown in Figure 12, the CDH6-targeting antibody drug conjugate NOV0712-sulfo-SPDB-DM4 exhibited target and dose-dependent cellular activity as indicated by inhibition of proliferation in CDH6 expressing cell lines (OVCAR3 and OVCAR8-CDH6+) compared to the non-expressing cell line (OVCAR8). No cellular activity was observed with non-conjugated NOV0712 antibody or a non-targeting IgG control. Marginal activity at concentrations understood to result in non-specific cytotoxicity were seen with a control IgG-sulfo-SPDB-DM4 conjugate. The cellular activity assay was performed and analyzed as disclosed in Example 9.

Example 15: In vivo efficacy of anti-CDH6 antibodies as SMCC-DM1 conjugates in a xenograft mouse model of ovarian cancer.

[00434] The anti-tumor activity of a selection of anti-CDH6 ADCs were evaluated in the OVCAR-3 ovarian xenograft model. Female NOD-scid-gamma mice were implanted subcutaneously on the right flank with 10×10^6 OVCAR-3 cells containing 50% Matrigel™ (BD Biosciences) in phosphate buffer solution (PBS). The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study 29 days post implantation with average tumor volume of 165 mm³. After being randomly assigned to one of seven groups (n = 4/group), mice were administered a single i.v. dose of PBS (10 ml/kg), a non-target isotype control hIgG1-SMCC-DM1 (10 mg/kg), or one of five anti-CDH6-SMCC-DM1 (10 mg/kg). Tumor volumes and body weights were measured twice weekly. Each anti-CDH6-SMCC-DM1 ADC elicited an inhibitory effect upon tumor growth following a single dose of 10mg/kg, when compared to control arms (Figure 13).

[00435] The anti-tumor activity of a second selection of anti-CDH6 ADCs were evaluated in the OVCAR-3 ovarian xenograft model. Female NOD-scid-gamma mice were implanted subcutaneously on the right flank with 10×10^6 OVCAR-3 cells containing 50% Matrigel™ (BD Biosciences, San Jose, CA) in PBS. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study 35 days post implantation with average tumor volume of 166 mm³. After being randomly assigned to one of seven groups (n = 4/group), mice were administered a single i.v. dose of PBS (10 ml/kg), a non-target isotype control hIgG1-SMCC-DM1 (10 mg/kg), or one of five anti-CDH6-SMCC-DM1 (10 mg/kg) plus NOV0712-SMCC-DM1 which had been dosed in the previous OVCAR3 triage. Tumor volumes and body weights were measured twice weekly. None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated (data not shown). Of the five anti-CDH6-SMCC-DM1, only NOV0690 elicited a response comparable to that of NOV0712-SMCC-DM1 (Figure 14).

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Example 16: In vivo efficacy of anti-CDH6 antibodies as SPDB-DM4 conjugates in a xenograft mouse model of ovarian cancer

[00436] The anti-tumor activity of a selection of anti-CDH6 ADCs were evaluated in the OVCAR-3 ovarian xenograft model. Female NOD-scid-gamma mice were implanted subcutaneously on the right flank with 10×10^6 OVCAR-3 cells containing 50% Matrigel™ (BD Biosciences, San Jose, CA) in PBS. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study 21 days post implantation with average tumor volume of 174 mm³. After being randomly assigned to one of nine groups (n = 5/group), mice were administered a single i.v. dose of PBS, (10 ml/kg), a non-target isotype control IgG1-SPDB-DM4 (5 mg/kg), a non-target isotype control IgG1-SMCC-DM1 (5 mg/kg), NOV0712-SMCC-DM1 (5 mg/kg), or one of seven anti-CDH6-SPDB-DM4 (5 mg/kg). Tumor volumes and body weights were measured 1-2 times weekly. None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated at this dosage (data not shown). Of all the anti-CDH6-SPDB-DM4 agents, NOV0712-SPDB-DM4 elicited the greatest anti-tumor effect with regression 70 days post dose. NOV0710-SPDB-DM4 was the second most active anti-CDH6-SPDB-DM4 in this study (Figure 15).

Example 17: In vivo efficacy of anti-CDH6 antibodies as SMCC-DM1 or SPDB-DM4 conjugates in a xenograft mouse model of ovarian cancer

[00437] The anti-tumor activity of a selection of anti-CDH6 ADC NOV0712 in different linker-payload formats were evaluated in the OVCAR-3 ovarian xenograft model. Female NOD-scid-gamma mice were implanted subcutaneously on the right flank with 10×10^6 OVCAR-3 cells containing 50% Matrigel™ (BD Biosciences, San Jose, CA) in PBS. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study 21 days post implantation with average tumor volume of 174 mm³. After being randomly assigned to one of five groups (n = 5/group), mice were administered a single i.v. dose of PBS, (10 ml/kg), a non-target isotype control IgG1-SPDB-DM4 (5 mg/kg), a non-target isotype control IgG1-SMCC-DM1 (5 mg/kg), NOV0712-SMCC-DM1 (5 mg/kg), or NOV0712-SPDB-DM4 (5 mg/kg). Tumor volumes and body weights were measured 1-2 times weekly. None of the mice showed a decrease in body weight (data not shown). Both L/P formats of NOV0712 elicited an antitumor effect, but the SPDB-DM4 format was much more potent leading to a durable regression for over 70 days post dose (Figure 16). Pharmacokinetic sampling indicates the two agents have similar PK profiles (data not shown).

Example 18: In vivo efficacy of anti-CDH6 ADCs with different linker/payload formats in a patient-derived primary tumor xenograft mouse model of ovarian cancer

[00438] The anti-tumor activity of different linker payload formats of the anti-CDH6 ADC NOV0712 were evaluated in CDH6 expressing primary (patient derived) ovarian tumors xenografted

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into mice. Human primary xenograft models were established by direct implantation of primary human tumor tissue subcutaneously into nu/nu nude mice. The resulting xenografts were serially passaged between 4 and 10 times prior to use in this study. Athymic nude mice were implanted with fragments of tumor approximately 27 mm³ in size, subcutaneously on the right flank. Mice were enrolled in the study 48 days post implant with a mean tumor volume of 178 mm³. After being randomized into one of eight groups (n = 5/group) Mice were administered a single i.v. dose every 14 days (Q14D), of PBS (10 ml/kg), a non-target isotype control hIgG1 linker payload format (-SMCC-DM1, -SPDB-DM4, -sulfo-SPDB-DM4), a NOV0712 linker payload format (-SMCC-DM1, -SPDB-DM4, -sulfo-SPDB-DM4) or NOV0712 as a naked antibody. All groups were dosed at 5 mg/kg. Tumor volumes and bodyweights were measured 1-2 times weekly. None of the mice showed a decrease in body weight (data not shown). NOV0712 as a naked antibody had no inhibitory effect upon tumor growth. NOV0712-SMCC-DM1 treatment did not result in regression in this model. Each cleavable format of NOV0712 resulted in regressions. NOV0712-sulfo-SPDB-DM4 at 5mg/kg Q14D (single dose every 14 days) was the most potent treatment arm with durable tumor regression lasting over 150 days post first dose (Figure 17).

Example 19: In vivo efficacy of anti-CDH6 ADCs with different linker/payload formats in a xenograft mouse model of ovarian cancer

[00439] The anti-tumor activity of a selection of anti-CDH6 ADCs were evaluated in the OVCAR-3 ovarian xenograft model. Female NOD-scid-gamma mice were implanted subcutaneously on the right flank with 10x10⁶ OVCAR-3 cells containing 50% Matrigel™ (BD Biosciences, San Jose CA) in PBS. The total injection volume containing cells in suspension was 200 µl. Mice were enrolled in the study 20 days post implantation with average tumor volume of 172 mm³. After being randomized into one of eight groups (n = 5/group) mice were administered a single i.v. dose of PBS (10 ml/kg), a non-target isotype control hIgG1-SPDB-DM4 (5 mg/kg), a non-target isotype control hIgG1-sulfo-SPDB-DM4 (5 mg/kg), NOV0712-SPDB-DM4 (1.25, 2.5 and 5 mg/kg), or NOV0712-sulfo-SPDB-DM4 (1.25, 2.5 and 5 mg/kg). Tumor volumes and bodyweights were measured 1-2 times weekly throughout the duration of the study. None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated (data not shown). NOV0712-sulfo-SPDB-DM4 was more efficacious than NOV0712-SPDB at both the 2.5 mg/kg and 5 mg/kg dose levels. A single 2.5 mg/kg dose of NOV0712-sulfo-SPDB-DM4 lead to regression. A single 5 mg/kg dose of each format led to regression, but the effect of the sulfo-SPDB-DM4 dose outlasted that of the -SPDB-DM4 dose. Overall these data show the sulfo-SPDB-DM4 format is the most active format for targeting CDH6 using ADCs out of the panel assessed (Figure 18).

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Example 20: In vivo efficacy of anti-CDH6 ADCs with different linker/payload formats in a patient-derived primary tumor xenograft mouse model of ovarian cancer

[00440] The anti-tumor activity of different linker payload formats of the anti-CDH6 ADC NOV0712 were evaluated in CDH6 expressing primary (patient derived) ovarian tumors xenografted into mice. Human primary xenograft models were established by direct implantation of primary human tumor tissue subcutaneously into nu/nu nude mice. The resulting xenografts were serially passaged between 4 and 10 times prior to use in this study. Athymic nude mice were implanted with fragments of tumor approximately 27 mm³ in size, subcutaneously on the right flank. Due to the variable latencies in the growth kinetics of the primary tumors, mice were entered into the study in a rolling fashion. Tumors were assigned to a group when upon reaching an appropriate volume, between 147 mm³ and 244 mm³ (the mean size at enrollment was 180 mm³). The first 5 mice were assigned to groups 1 through 5, the next 5 mice were assigned to groups 5 through 1, and so on until 5 groups (n=5/group) were filled. Upon enrollment each mouse was administered with an i.v. dose every 2 weeks (Q14D), of PBS(10 ml/kg), a non-target isotype control hlgG1-SMCC-DM1 (5 mg/kg), NOV0712-SMCC-DM1 (5 mg/kg), non-target isotype control hlgG1-sulfo-SPDB-DM4 (2.5 mg/kg) or NOV0712-sulfo-SPDB-DM4 (2.5 mg/kg). Tumor volumes and bodyweights were measured twice weekly. None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated (data not shown). NOV0712-SMCC-DM1 5mg/kg Q14D is able to reduce tumor growth in this model. NOV0712-sulfo-SPDB-DM4 2.5mg/kg Q14D is efficacious in halting tumor growth in this model for 42 days (Figure 19).

Example 21: In vivo efficacy of an anti-CDH6 ADC in a panel of patient-derived primary tumor xenograft mouse models of ovarian cancer.

[00441] The anti-tumor activity of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4) was assessed in a panel of 28 ovarian primary tumor xenograft (PTX) models in a 1x1x1 design (1PTX x 1 animal x 1 treatment). Human primary xenograft models were established by direct implantation of primary human tumor tissue subcutaneously into nu/nu nude mice. The resulting xenografts were serially passaged between 4 and 10 times prior to use in this study. Female nude mice were implanted subcutaneously in the axillary region, with a 3 x 3 x 3 mm tumor fragment using a 12 gauge trocar. Once a tumor measured 200-250 mm³ the mouse was enrolled into the study and treatment started. Upon enrollment each mouse was administered with an i.v. dose every 2 weeks (Q14D), of NOV0712-sulfo-SPDB-DM4 (5 mg/kg). None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated (data not shown). Tumor volumes and bodyweights were measured twice weekly. For visualization of anti-tumor activity, the percent tumor volume change over time was plotted using Tibco Spotfire (Tibco Software, Palo Alto, CA).

[00442] CDH6 expression in the PTX samples was assessed using immunohistochemistry (IHC) on samples from untreated PTX tumors. Specifically, at sacrifice, tumors were immediately

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excised, collected into histology cassettes and fixed in 10% buffered formalin for 24 hours. Cassettes were then transferred into 70% EtOH and processed and embedded in paraffin using routine histological procedures. Experimental FFPE blocks were cut at 3.5 μ m as whole sections on slides. A rabbit polyclonal anti human CDH6 antibody obtained from Sigma-Aldrich (Cat # HPA007047, Sigma Aldrich, St. Louis, MO) and used as the primary immunohistochemistry (IHC) antibody. The antibody was detected using Ventana Biotin-free DAB Detection Systems on the Ventana DISCOVERY XT Biomarker Platform (Tucson, AZ).

[00443] The optimized protocol included standard exposure to Ventana Cell Conditioning #1 antigen retrieval reagent (Cat # 950-124). The primary antibody was diluted to a concentration of 1:200 in DAKO Cytomation Antibody Diluent (Cat # S0809), applied in 100 μ l volume and incubated for 60 minutes at 37°C. Subsequently incubation with Ventana OmniMap prediluted HRP-conjugated anti-rabbit secondary antibody (Cat # 760-4311) was performed for 4 minutes. The secondary antibody was then detected using the ChromoMap DAB kit (Cat # 760-159) and slides were counterstained for 4 minutes with Ventana Hematoxylin (Cat # 760-2021), followed by Ventana Bluing Reagent (Cat # 760-2037) for 4 minutes. Slides were dehydrated in increasing concentrations of ethanol (95-100%), then in xylenes, followed by coverslipping. Coverslipped slides were evaluated by light microscopy and scanned by Leica/Aperio ScanScope slide scanner (Vista, CA). Scanned images of the stained slides were launched in Indica Labs HALO (Cortices, NM) opening from integrated Leica eSlide Manager/Aperio Spectrum (Vista, CA). Digital images were viewed, annotated and analyzed using Area Quantification Algorithm (Area Quantification v1.0) either with Classifier Module (CDH6 Loose Tumor 2, CDH6 Neg Tumor 2 & CDH6 Tumor & CDH6 tumor2) for tumor detection or without Classifier Module. Image analysis algorithms were used to detect any intensity.

[00444] As illustrated in Figure 20, efficacy of anti-CDH6 ADC was observed in the PTX models, particularly amongst tumor models featuring greater than twenty percent CDH6 positive tumor area by IHC. Of special note, 11/28 (39%) of the PTX models responded with complete tumor regression lasting in excess of 150 days.

Example 22: In vivo efficacy of an anti-CDH6 ADC dosed either at 2.5 mg/kg i.v., Q14D or 5 mg/kg i.v. Q14D in a patient-derived primary tumor xenograft mouse model of renal cancer.

[00445] The anti-tumor activity of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4, dosed either at 5mg/kg Q14D or 2.5 mg/kg, Q14D) was assessed in a renal primary tumor xenograft (PTX) model. Human primary xenograft models were established by direct implantation of primary human tumor tissue subcutaneously into nu/nu nude mice. The resulting xenografts were serially passaged between 4 and 10 times prior to use in this study. Female nude mice were implanted subcutaneously in the axillary region, with a 3 x 3 x 3 mm tumor fragment using a 12 gauge trocar. Animals were randomized into treatment groups on day 20 following tumor fragment implantation, when the average tumor volume was 195 mm³ (range 115-282 mm³). Treatments were initiated on Day 20. Anti-tumor

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activity was determined on day 53 post tumor cell implant, 33 days post initiation of treatment, the last day when all animals remained on study. A non-targeting ADC was included as control for determining CDH6-specific anti-tumor activity. None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated (data not shown). As with the ovarian cancer models, efficacy of anti-CDH6 ADC was observed in renal PTX models featuring greater than twenty percent CDH6 positive tumor area by IHC. As shown in Figure 21, anti-CDH6 ADC treatment was efficacious against primary human tumors in a renal PTX model.

Example 23: In vivo efficacy of an anti-CDH6 ADC dosed either at 2.5 mg/kg, Q14D i.v. or 5 mg/kg i.v. Q14D in a patient-derived primary tumor xenograft mouse model of renal cancer.

[00446] The anti-tumor activity of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4) was assessed in a renal primary tumor xenograft (PTX) model. Human primary xenograft models were established by direct implantation of primary human tumor tissue subcutaneously into nu/nu mice. The resulting xenografts were serially passaged between 4 and 10 times prior to use in this study. Female nude mice were implanted subcutaneously in the axillary region, with a 3 x 3 x 3 mm tumor fragment using a 12 gauge trocar. Animals were randomized into treatment groups on day 14 following tumor fragment implantation, when the average tumor volume was 160mm³ (range 113-245 mm³). Treatments were initiated on Day 14. Anti-tumor activity was determined on day 50 post tumor cell implant. None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated (data not shown). As with the ovarian cancer models, efficacy of anti-CDH6 ADC was observed in renal PTX models featuring greater than twenty percent CDH6 positive tumor area by IHC. As shown in Figure 22, anti-CDH6 ADC treatment was efficacious against primary human tumors in a renal PTX model.

Example 24: In vivo efficacy of an anti-CDH6 ADC dosed either at 2.5 mg/kg, Q14D or 5 mg/kg Q14D in a patient-derived primary tumor xenograft mouse model of renal cancer.

[00447] The anti-tumor activity of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4) was assessed in a renal primary tumor xenograft (PTX) model. Human primary xenograft models were established by direct implantation of primary human tumor tissue subcutaneously into nu/nu nude mice. The resulting xenografts were serially passaged between 4 and 10 times prior to use in this study. Female nude mice were implanted subcutaneously in the axillary region, with a 3 x 3 x 3 mm tumor fragment using a 12 gauge trocar. Animals were randomized into treatment groups on day 19 following tumor fragment implantation, when the average tumor volume was 176 mm³ (range 123-267 mm³). Treatments were initiated on Day 19. Anti-tumor activity was determined on day 49 post tumor cell implant. None of the mice showed a decrease in body weight, indicating that the ADC was well tolerated (data not shown). As with the ovarian cancer models, efficacy of anti-CDH6 ADC was observed in renal PTX models featuring greater than twenty percent CDH6 positive tumor area by

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IHC. As shown in Figure 23, anti-CDH6 ADC treatment was efficacious against primary human tumors in a renal PTX model at both the 5 mg/kg and 2.5 mg/kg dosage.

Example 25: Anti-CDH6 ADC in combination therapy

[00448] Anti CDH6 ADCs can be combined with small molecule inhibitors or other antibodies. Using either the Chalice software (Zalions, Cambridge MA) or ComboExplorer application (Novartis, Basel CH), the response of the combination is compared to its single agents, against the widely used Loewe model for drug-with-itself dose-additivity (Lehar et al. Nat. Biotechnol. (2009) 27: 659-666; Zimmermann et al., Drug Discov. Today (2007) 12: 34-42). Excess inhibition compared to additivity can be plotted as a full dose-matrix chart to visualize the drug concentrations where synergies occur. Table 18 shows several anti-CDH6 ADC/compound combinations.

[00449] Cell viability can be determined by measuring cellular ATP content using the CellTiter Glo® luminescence assay (Promega, Madison WI). One day before drug addition, 250-500 cells are plated into 384-well plates (Greiner, Monroe, NC) in 20 µl growth media. Cells are then incubated for 120 h with various concentrations of anti-CDH6 ADC (Table 18), as a single agent, single agent compounds or anti-CDH6 ADC/compound combinations before CellTiter Glo® reagent is added to each well and luminescence recorded on an Envision® plate reader (Perkin Elmer, Waltham MA). Luminescence values are used to calculate the inhibition of cell viability relative to DMSO-treated cells (0% inhibition).

Table 18

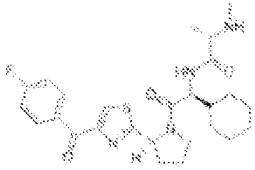
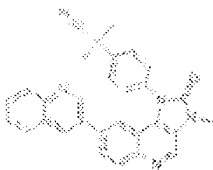
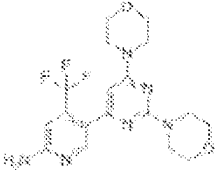
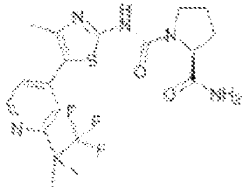
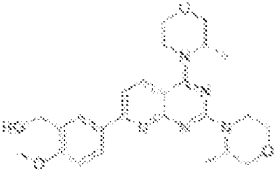
anti-CDH6 ADC combinations

<u>Target of compound</u>	<u>anti-CDH6 ADC in combination with:</u>	<u>Structure</u>
RTK:	Glivec®	Imatinib
RTK:	Sutent®	Sumatinib

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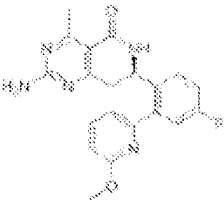
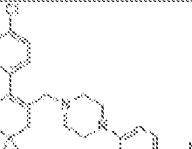
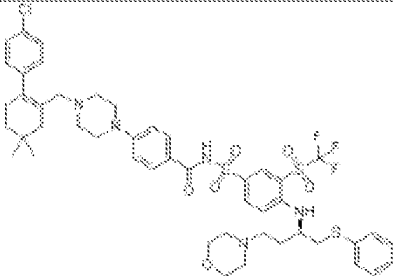
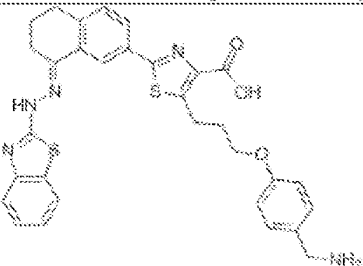
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RTKi	Votrient®	pazopanib
IAPi	NVP-LCL161	
PI3K fam.	NVP-BEZ235	
p110 PI3K/	NVP-BKM120	
PI3K	NVP-BYL719	
mTOR/ (cat.)	NVP-CCG168	

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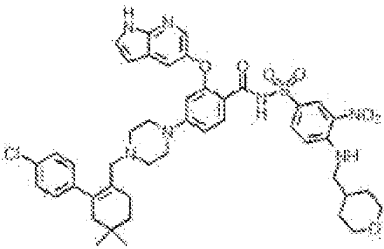
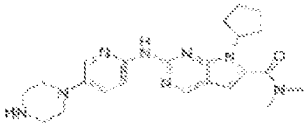
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mTOR/ (allo.)	Afinitor®	Everolimus
		
HSP90α	NVP-HSP900	
		
JAK2	NVP-BV808	
		
BCL2/BCL-Xl	ABT263	
		
BCL-Xl	WEHI-539	
		

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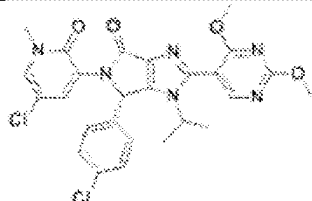
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BCL2	ABT199	
HDAC	Farydak®	panobinostat
		
CDH4/6	NVP-LEE011	
MEK	Mekinist®	Trametinib
RAF	Tafinlar®	Dabrafenib
DNA	Paraplatin®	carboplatin

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HDM2	NVP-HDM201	
Tubulin	Taxol®	paclitaxel
HER3	NVP-LJM716	anti-HER3 antibody (Garner et al., Cancer Res 2013 73(19):6024-35)

[00450] Figure 24 is an example of anti-CDH6 ADC in combination with small molecule inhibitors. SNU-8 ovarian cancer cells were grown as described in Example 12 and treated with titrations of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4) and either a Bcl2/Bcl-Xl inhibitor (ABT-263), a Bcl-Xl inhibitor (WEHI-539), an IAP inhibitor (NVP-LCL161) or a MEK inhibitor (trametinib). Potent synergy is seen with an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4) in combination with Bcl2/Bcl-Xl, Bcl-Xl, MEK and IAP inhibitors. Self-self combinations of ADC, for example, (CDH6-ADC + CDH6-ADC) or self-self combinations of a small molecule, for example, (NVP-LCL161 + NVP-LCL161) were included to distinguish dose additivity from true synergy and these scores are reported in Figure 24. As Figure 24 indicates, the CDH6-ADC plus small molecule combinations feature significantly higher Loewe Synergy Scores compared to the self-self combinations.

[00451] This data indicates that the combination of an ADC with an anti-mitotic payload (for example, sulfo-SPDB-DM4, SPDB-DM4, SMCC-DM1) or other payloads with a pro-apoptotic small molecule inhibitor (for example, ABT-261, ABT-199, WEHI-539 or IAP inhibitors like NVP-LCL161) or MAPK pathway inhibitors (i.e. trametinib) results in more effective execution of apoptosis following initial G2/M arrest or damage induced by the anti-mitotic/cytotoxic ADC payload alone.

[00452] In addition, CDH6-ADC combinations can further be assessed by in vivo combination treatment. CDH6-ADCs can synergize with immuno-modulatory agents targeting the PD/PD-L1 pathway such as Keytruda® (pembrolizumab) or Opdivo® (nivolumab). In summary, the anti-CDH6

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ADCs disclosed herein can exert synergistic effects when used in combination with other molecules to lead to more options for treatment.

Example 26: CDH6 expression in tumor indications

[00453] CDH6 is over-expressed in several tumor indications including ovarian cancer, renal cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma. CDH6 mRNA expression was assessed using the Affymetrix GeneChip® platform (Santa Clara, CA). Specifically, a Novartis-internal mRNA expression database comprising data on human tumor and normal tissue samples was queried using the CDH6 probeset 214803_at and the data were plotted using Tibco Spotfire (Palo Alto, CA). Each point on the graph represents an individual tumor or normal tissue sample. As illustrated in Figure 25, CDH6 features a restricted normal tissue expression and is significantly overexpressed in ovarian cancer, renal cancer, CNS cancers, soft tissue cancers and thyroid cancer and cholangiocarcinoma.

[00454] CDH6 expression was further assessed in a collection of human primary tumor samples by immuno-histochemistry using the protocol described in Example 21. As shown in Figure 26, CDH6 protein expression was observed in the majority of ovarian cancer and renal cancer samples. CDH6 expression was also frequently detected in endometrial cancer as well as cholangiocarcinoma. Interestingly, CDH6 expression within cholangiocarcinoma was enriched amongst the intra-hepatic sub-type.

[00455] It is understood that the examples and aspects described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

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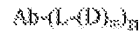
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CLAIMS

What is claimed is:

1. An antibody drug conjugate of the formula



or a pharmaceutically acceptable salt thereof; wherein

Ab is an antibody or antigen binding fragment thereof that specifically binds to an epitope of human CDH6;

L is a linker;

D is a drug moiety;

m is an integer from 1 to 8; and

n is an integer from 1 to 10.

2. The antibody drug conjugate of claim 1, wherein said n is 3 or 4.

3. The antibody drug conjugate of claim 1 or 2, wherein said antibody or antigen binding fragment thereof specifically binds the extracellular domain of CDH6, comprising the amino acid sequence of SEQ ID NO:4.

4. The antibody drug conjugate of claim 1 or 2, wherein said antibody or antigen binding fragment specifically binds to an epitope of human CDH6 comprising the amino acid sequence of SEQ ID NO:534.

5. The antibody drug conjugate of any of claims 1-4, wherein said antibody or antigen binding fragment thereof comprises:

(i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;

(ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;

(iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and

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a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;

(iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;

(v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:14, (b) a LCDR2 of SEQ ID NO:15, (c) a LCDR3 of SEQ ID NO:16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:17, (e) a HCDR2 of SEQ ID NO:18, and (f) a HCDR3 of SEQ ID NO:19;

(vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;

(vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;

(viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;

(ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

(x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;

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(xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO:100; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:101, (e) a HCDR2 of SEQ ID NO:102, and (f) a HCDR3 of SEQ ID NO:103;

(xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:112, (b) a LCDR2 of SEQ ID NO:113, (c) a LCDR3 of SEQ ID NO:114; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:115, (e) a HCDR2 of SEQ ID NO:116, and (f) a HCDR3 of SEQ ID NO:117;

(xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:126, (b) a LCDR2 of SEQ ID NO:127, (c) a LCDR3 of SEQ ID NO:128; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:129, (e) a HCDR2 of SEQ ID NO:130, and (f) a HCDR3 of SEQ ID NO:131;

(xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:140, (b) a LCDR2 of SEQ ID NO:141, (c) a LCDR3 of SEQ ID NO:142; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:143, (e) a HCDR2 of SEQ ID NO:144, and (f) a HCDR3 of SEQ ID NO:145;

(xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:154, (b) a LCDR2 of SEQ ID NO:155, (c) a LCDR3 of SEQ ID NO:156; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:157, (e) a HCDR2 of SEQ ID NO:158, and (f) a HCDR3 of SEQ ID NO:159;

(xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:168, (b) a LCDR2 of SEQ ID NO:169, (c) a LCDR3 of SEQ ID NO:170; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:171, (e) a HCDR2 of SEQ ID NO:172, and (f) a HCDR3 of SEQ ID NO:173;

(xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:182, (b) a LCDR2 of SEQ ID NO:183, (c) a LCDR3 of SEQ ID NO:184; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:185, (e) a HCDR2 of SEQ ID NO:186, and (f) a HCDR3 of SEQ ID NO:187;

(xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:196, (b) a LCDR2 of SEQ ID NO:197, (c) a LCDR3 of SEQ ID NO:198; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

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(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

6. The antibody drug conjugate of any of the preceding claims in which at least one amino acid within a CDR is substituted by a corresponding residue of a corresponding CDR of another anti-CDH6 antibody in Table 5 or 6.

7. The antibody drug conjugate of any of the preceding claims in which one or two amino acids within a CDR have been modified, deleted or substituted.

8. The antibody drug conjugate of any of claims 1-4, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain variable region (vH) that comprises SEQ ID NO: 230, and a light chain variable region (vL) that comprises SEQ ID NO:231;

(ii) a heavy chain variable region (vH) that comprises SEQ ID NO: 216, and a light chain variable region (vL) that comprises SEQ ID NO:217;

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(iii) a heavy chain variable region (vH) that comprises SEQ ID NO: 272, and a light chain variable region (vL) that comprises SEQ ID NO:273;

(iv) a heavy chain variable region (vH) that comprises SEQ ID NO:314, and a light chain variable region (vL) that comprises SEQ ID NO:315;

(v) a heavy chain variable region (vH) that comprises SEQ ID NO:20, and a light chain variable region (vL) that comprises SEQ ID NO:21;

(vi) a heavy chain variable region (vH) that comprises SEQ ID NO: 34, and a light chain variable region (vL) that comprises SEQ ID NO:35;

(vii) a heavy chain variable region (vH) that comprises SEQ ID NO:48, and a light chain variable region (vL) that comprises SEQ ID NO:49;

(viii) a heavy chain variable region (vH) that comprises SEQ ID NO:62, and a light chain variable region (vL) that comprises SEQ ID NO:63;

(ix) a heavy chain variable region (vH) that comprises SEQ ID NO:76, and a light chain variable region (vL) that comprises SEQ ID NO:77;

(x) a heavy chain variable region (vH) that comprises SEQ ID NO:90, and a light chain variable region (vL) that comprises SEQ ID NO:91;

(xi) a heavy chain variable region (vH) that comprises SEQ ID NO:104, and a light chain variable region (vL) that comprises SEQ ID NO:105;

(xii) a heavy chain variable region (vH) that comprises SEQ ID NO: 118, and a light chain variable region (vL) that comprises SEQ ID NO:119;

(xiii) a heavy chain variable region (vH) that comprises SEQ ID NO: 132, and a light chain variable region (vL) that comprises SEQ ID NO:133;

(xiv) a heavy chain variable region (vH) that comprises SEQ ID NO: 146, and a light chain variable region (vL) that comprises SEQ ID NO:147;

(xv) a heavy chain variable region (vH) that comprises SEQ ID NO:160, and a light chain variable region (vL) that comprises SEQ ID NO:161;

(xvi) a heavy chain variable region (vH) that comprises SEQ ID NO: 174, and a light chain variable region (vL) that comprises SEQ ID NO:175;

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(xvii) a heavy chain variable region (vH) that comprises SEQ ID NO: 188, and a light chain variable region (vL) that comprises SEQ ID NO:189;

(xviii) a heavy chain variable region (vH) that comprises SEQ ID NO: 202, and a light chain variable region (vL) that comprises SEQ ID NO:203;

(xix) a heavy chain variable region (vH) that comprises SEQ ID NO: 244, and a light chain variable region (vL) that comprises SEQ ID NO:245;

(xx) a heavy chain variable region (vH) that comprises SEQ ID NO:258, and a light chain variable region (vL) that comprises SEQ ID NO:259;

(xxi) a heavy chain variable region (vH) that comprises SEQ ID NO:286, and a light chain variable region (vL) that comprises SEQ ID NO:287;

(xxii) a heavy chain variable region (vH) that comprises SEQ ID NO:300, and a light chain variable region (vL) that comprises SEQ ID NO:301; or

(xxiii) a heavy chain variable region (vH) that comprises SEQ ID NO:328, and a light chain variable region (vL) that comprises SEQ ID NO:329.

9. The antibody drug conjugate of any of claims 1-4, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain that comprises SEQ ID NO: 234, and a light chain that comprises SEQ ID NO:235;

(ii) a heavy chain that comprises SEQ ID NO: 220, and a light chain that comprises SEQ ID NO:221

(iii) a heavy chain that comprises SEQ ID NO: 276, and a light chain that comprises SEQ ID NO:277;

(iv) a heavy chain that comprises SEQ ID NO:318, and a light chain that comprises SEQ ID NO:319;

(v) a heavy chain that comprises SEQ ID NO:24, and a light chain that comprises SEQ ID NO:25;

(vi) a heavy chain that comprises SEQ ID NO: 38, and a light chain that comprises SEQ ID NO:39;

(vii) a heavy chain that comprises SEQ ID NO:52, and a light chain that comprises SEQ ID NO:53;

(viii) a heavy chain that comprises SEQ ID NO:66, and a light chain that comprises SEQ ID NO:67;

(ix) a heavy chain that comprises SEQ ID NO:80, and a light chain that comprises SEQ ID NO:81;

(x) a heavy chain that comprises SEQ ID NO:94, and a light chain that comprises SEQ ID NO:95;

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- (xi) a heavy chain that comprises SEQ ID NO: 108, and a light chain that comprises SEQ ID NO: 109;
 - (xii) a heavy chain that comprises SEQ ID NO: 122, and a light chain that comprises SEQ ID NO: 123;
 - (xiii) a heavy chain that comprises SEQ ID NO: 136, and a light chain that comprises SEQ ID NO: 137;
 - (xiv) a heavy chain that comprises SEQ ID NO: 150, and a light chain that comprises SEQ ID NO: 151;
 - (xv) a heavy chain that comprises SEQ ID NO: 164, and a light chain that comprises SEQ ID NO: 165;
 - (xvi) a heavy chain that comprises SEQ ID NO: 178, and a light chain that comprises SEQ ID NO: 179;
 - (xvii) a heavy chain that comprises SEQ ID NO: 192, and a light chain that comprises SEQ ID NO: 193;
 - (xviii) a heavy chain that comprises SEQ ID NO: 206, and a light chain that comprises SEQ ID NO: 207;
 - (xix) a heavy chain that comprises SEQ ID NO: 248, and a light chain that comprises SEQ ID NO: 249;
 - (xx) a heavy chain that comprises SEQ ID NO: 262, and a light chain that comprises SEQ ID NO: 263;
 - (xxi) a heavy chain that comprises SEQ ID NO: 290, and a light chain that comprises SEQ ID NO: 291;
 - (xxii) a heavy chain that comprises SEQ ID NO: 304, and a light chain that comprises SEQ ID NO: 305; or
 - (xxiii) a heavy chain that comprises SEQ ID NO: 332, and a light chain that comprises SEQ ID NO: 333.
10. The antibody drug conjugate of any of the preceding claims that retains at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% identity over either the variable light or variable heavy region.
11. The antibody drug conjugate of any of the preceding claims wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human engineered antibody, a human antibody, a single chain antibody(scFv) or an antibody fragment.

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12. The antibody drug conjugate of any one of claims 1-11, wherein said linker (L) is selected from the group consisting of a cleavable linker, a non-cleavable linker, a hydrophilic linker, a precharged linker and a dicarboxylic acid based linker.

13. The antibody drug conjugate of claim 12, wherein the linker is derived from a cross-linking reagent selected from the group consisting of: N-succinimidyl 4-(2-pyridyldithio)2-sulfo-butanate (sulfo-SPDB), N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC) or 2,5-dioxypyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetramazaheptadecan-1-oate (CX1-1).

14. The antibody drug conjugate of claim 13, wherein said linker is derived from N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanate (sulfo-SPDB).

15. The antibody drug conjugate of any one of claims 1-14, wherein said drug moiety (D) is selected from a group consisting of: a maytansinoid, a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHER inhibitor.

16. The antibody drug conjugate of claim 15, wherein the drug moiety is a maytansinoid.

17. The antibody drug conjugate of claim 16, wherein the maytansinoid is N(2')-deacetyl-N2-(4-mercapto-4-methyl-1-oxopentyl)-maytansine (DM4) or N(2')-deacetyl-N(2')-(3-mercapto-4-oxopropyl)-maytansine (DM1).

18. The antibody drug conjugate of any of the preceding claims in combination with another therapeutic agent.

19. The antibody drug conjugate of any of the preceding claims in combination with a therapeutic agent listed in Table 18.

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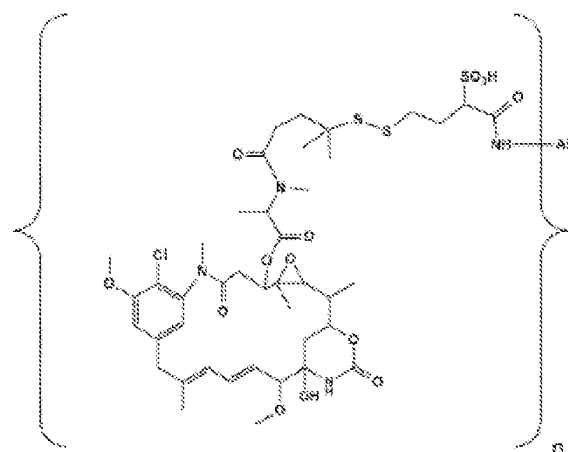
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20. The antibody drug conjugate of any of the preceding claims in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.

21. The antibody drug conjugate of any of the preceding claims in combination with an immune modulatory molecule.

22. An antibody drug conjugate of the formula:



Ab-sulfo-SPDB-DM4

or a pharmaceutically acceptable salt thereof; wherein;

Ab is an antibody or antigen binding fragment thereof that specifically binds to human CDH6, and n is an integer from 1 to 10.

23. The antibody drug conjugate of claim 22, wherein said antibody or antigen binding fragment specifically binds to an epitope of human CDH6 at (SEQ ID NO:4).

24. The antibody drug conjugate of claim 22, wherein said antibody or antigen binding fragment thereof specifically binds human CDH6 at (SEQ ID NO:534).

25. The antibody drug conjugate of claim 22, wherein said Ab is an antibody or antigen binding fragment thereof comprises:

(i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and

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a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;

(ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;

(iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;

(iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;

(v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:14, (b) a LCDR2 of SEQ ID NO:15, (c) a LCDR3 of SEQ ID NO:16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:17, (e) a HCDR2 of SEQ ID NO:18, and (f) a HCDR3 of SEQ ID NO:19;

(vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;

(vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;

(viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;

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(ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

(x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;

(xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO:100; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:101, (e) a HCDR2 of SEQ ID NO:102, and (f) a HCDR3 of SEQ ID NO:103;

(xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:112, (b) a LCDR2 of SEQ ID NO:113, (c) a LCDR3 of SEQ ID NO:114; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:115, (e) a HCDR2 of SEQ ID NO:116, and (f) a HCDR3 of SEQ ID NO:117;

(xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:126, (b) a LCDR2 of SEQ ID NO:127, (c) a LCDR3 of SEQ ID NO:128; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:129, (e) a HCDR2 of SEQ ID NO:130, and (f) a HCDR3 of SEQ ID NO:131;

(xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:140, (b) a LCDR2 of SEQ ID NO:141, (c) a LCDR3 of SEQ ID NO:142; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:143, (e) a HCDR2 of SEQ ID NO:144, and (f) a HCDR3 of SEQ ID NO:145;

(xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:154, (b) a LCDR2 of SEQ ID NO:155, (c) a LCDR3 of SEQ ID NO:156; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:157, (e) a HCDR2 of SEQ ID NO:158, and (f) a HCDR3 of SEQ ID NO:159;

(xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:168, (b) a LCDR2 of SEQ ID NO:169, (c) a LCDR3 of SEQ ID NO:170; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:171, (e) a HCDR2 of SEQ ID NO:172, and (f) a HCDR3 of SEQ ID NO:173;

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(xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:182, (b) a LCDR2 of SEQ ID NO:183, (c) a LCDR3 of SEQ ID NO:184; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:185, (e) a HCDR2 of SEQ ID NO:186, and (f) a HCDR3 of SEQ ID NO:187;

(xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:196, (b) a LCDR2 of SEQ ID NO:197, (c) a LCDR3 of SEQ ID NO:198; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

26. The antibody drug conjugate of any of the preceding claims in which at least one amino acid within a CDR is substituted by a corresponding residue of a corresponding CDR of another anti-CDH6 antibody in Table 5 or 6.

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27. The antibody drug conjugate of any of the preceding claims in which one or two amino acids within a CDR have been modified, deleted or substituted.

28. The antibody drug conjugate of claim 22, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain variable region (vH) that comprises SEQ ID NO: 230, and a light chain variable region (vL) that comprises SEQ ID NO:231;

(ii) a heavy chain variable region (vH) that comprises SEQ ID NO: 216, and a light chain variable region (vL) that comprises SEQ ID NO:217;

(iii) a heavy chain variable region (vH) that comprises SEQ ID NO: 272, and a light chain variable region (vL) that comprises SEQ ID NO:273;

(iv) a heavy chain variable region (vH) that comprises SEQ ID NO:314, and a light chain variable region (vL) that comprises SEQ ID NO:315;

(v) a heavy chain variable region (vH) that comprises SEQ ID NO:20, and a light chain variable region (vL) that comprises SEQ ID NO:21;

(vi) a heavy chain variable region (vH) that comprises SEQ ID NO: 34, and a light chain variable region (vL) that comprises SEQ ID NO:35;

(vii) a heavy chain variable region (vH) that comprises SEQ ID NO:48, and a light chain variable region (vL) that comprises SEQ ID NO:49;

(viii) a heavy chain variable region (vH) that comprises SEQ ID NO:62, and a light chain variable region (vL) that comprises SEQ ID NO:63;

(ix) a heavy chain variable region (vH) that comprises SEQ ID NO:76, and a light chain variable region (vL) that comprises SEQ ID NO:77;

(x) a heavy chain variable region (vH) that comprises SEQ ID NO:90, and a light chain variable region (vL) that comprises SEQ ID NO:91;

(xi) a heavy chain variable region (vH) that comprises SEQ ID NO:104, and a light chain variable region (vL) that comprises SEQ ID NO:105;

(xii) a heavy chain variable region (vH) that comprises SEQ ID NO: 118, and a light chain variable region (vL) that comprises SEQ ID NO:119;

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(xiii) a heavy chain variable region (vH) that comprises SEQ ID NO: 132, and a light chain variable region (vL) that comprises SEQ ID NO:133;

(xiv) a heavy chain variable region (vH) that comprises SEQ ID NO: 146, and a light chain variable region (vL) that comprises SEQ ID NO:147;

(xv) a heavy chain variable region (vH) that comprises SEQ ID NO:160, and a light chain variable region (vL) that comprises SEQ ID NO:161;

(xvi) a heavy chain variable region (vH) that comprises SEQ ID NO: 174, and a light chain variable region (vL) that comprises SEQ ID NO:175;

(xvii) a heavy chain variable region (vH) that comprises SEQ ID NO: 188, and a light chain variable region (vL) that comprises SEQ ID NO:189;

(xviii) a heavy chain variable region (vH) that comprises SEQ ID NO: 202, and a light chain variable region (vL) that comprises SEQ ID NO:203;

(xix) a heavy chain variable region (vH) that comprises SEQ ID NO: 244, and a light chain variable region (vL) that comprises SEQ ID NO:245;

(xx) a heavy chain variable region (vH) that comprises SEQ ID NO:258, and a light chain variable region (vL) that comprises SEQ ID NO:259;

(xxi) a heavy chain variable region (vH) that comprises SEQ ID NO:286, and a light chain variable region (vL) that comprises SEQ ID NO:287;

(xxii) a heavy chain variable region (vH) that comprises SEQ ID NO:300, and a light chain variable region (vL) that comprises SEQ ID NO:301; or

(xxiii) a heavy chain variable region (vH) that comprises SEQ ID NO:328, and a light chain variable region (vL) that comprises SEQ ID NO:329.

29. The antibody drug conjugate of any of the preceding claims that retains at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% identity over either the variable light or variable heavy region.

30. The antibody drug conjugate of any of claims 22-29, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a human engineered antibody, a human antibody, a single chain antibody(scFv) or an antibody fragment.

31. The antibody drug conjugate of any of claims 22-29, wherein said n is an integer from 2 to 8.

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32. The antibody drug conjugate of any of claims 22-29, wherein said n is an integer from 3 to 4.
33. The antibody drug conjugate of any of claims 22-32 in combination with another therapeutic agent.
34. The antibody drug conjugate of any of claims 22-32 in combination with a therapeutic agent listed in Table 18.
35. The antibody drug conjugate of any of the preceding claims in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.
36. The antibody drug conjugate of any of the preceding claims in combination with an immune modulatory molecule.
37. A pharmaceutical composition comprising the antibody drug conjugate of any one of claims 1-32 and a pharmaceutically acceptable carrier.
38. The pharmaceutical composition of claim 37 wherein said composition is prepared as a lyophilisate.
39. A method of treating an CDH6 positive cancer in a patient in need thereof, comprising administering to said patient the antibody drug conjugate of any of claims 1-32, or the pharmaceutical composition of claims 37-38.
40. The method of claim 39, wherein said cancer is selected from the group consisting of: ovarian cancer, renal cancer, hepatic cancer, soft tissue cancer, CNS cancers, thyroid cancer and cholangiocarcinoma.
41. The method of claim 39, wherein the antibody drug conjugate or the pharmaceutical composition is administered in combination with another therapeutic agent.
42. The method of claim 39, wherein the antibody drug conjugate or the pharmaceutical composition is administered in combination with a therapeutic listed in Table 18.
43. The method of claim 39, wherein the antibody drug conjugate or the pharmaceutical composition is administered in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.
44. The method of claim 39, wherein the antibody drug conjugate or the pharmaceutical composition is administered in combination with an immune modulatory molecule.

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45. The antibody drug conjugate of any of claims 1-32 for use as a medicament.
46. The antibody drug conjugate of any of claims 1-32, or the pharmaceutical composition of any of claims 37-38, for use in the treatment of a CDH6 positive cancer.
47. The antibody drug conjugate of claim 45 administered in combination with another therapeutic agent.
48. The antibody drug conjugate of claim 45 administered in combination with a therapeutic agent listed in Table 18.
49. The antibody drug conjugate of claim 45 in combination with a BCL2 inhibitor, a BCL-XL inhibitor, a BCL2/BCL-XL inhibitor, an IAP inhibitor or a MEK inhibitor.
50. The antibody drug conjugate of claim 45 in combination with an immune modulatory molecule.
51. A nucleic acid that encodes the antibody or antigen binding fragment of any of claims 1-30.
52. A vector comprising the nucleic acid of claim 51.
53. A host cell comprising the vector according to claim 52.
54. A process for producing an antibody or antigen binding fragment comprising cultivating the host cell of claim 53 and recovering the antibody from the culture.
55. A process for producing an anti-CDH6 antibody drug conjugate, the process comprising:
(a) chemically linking N-succinimidyl-4-(2-pyridyldithio)2-sulfo-butanate (sulfo-SPDB) to a drug moiety DM-4;
(b) conjugating said linker-drug to the antibody recovered from the cell culture of claim 55; and
(c) purifying the antibody drug conjugate.
56. The antibody drug conjugate made according to claim 55 having an average maytansinoid to antibody ratio (MAR), measured with a UV spectrophotometer, about 3.5.
57. An antibody or antigen binding fragment thereof that comprises:
(i) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:224, (b) a LCDR2 of SEQ ID NO:225, (c) a LCDR3 of SEQ ID NO:226; and
a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 227, (e) a HCDR2 of SEQ ID NO: 228, and (f) a HCDR3 of SEQ ID NO:229;

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(ii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:210, (b) a LCDR2 of SEQ ID NO:211, (c) a LCDR3 of SEQ ID NO:212; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:213, (e) a HCDR2 of SEQ ID NO: 214, and (f) a HCDR3 of SEQ ID NO:215;

(iii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:266, (b) a LCDR2 of SEQ ID NO:267, (c) a LCDR3 of SEQ ID NO:268; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO: 269, (e) a HCDR2 of SEQ ID NO:270, and (f) a HCDR3 of SEQ ID NO: 271;

(iv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:308, (b) a LCDR2 of SEQ ID NO:309, (c) a LCDR3 of SEQ ID NO:310; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:311, (e) a HCDR2 of SEQ ID NO:312, and (f) a HCDR3 of SEQ ID NO:313;

(v) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:14, (b) a LCDR2 of SEQ ID NO:15, (c) a LCDR3 of SEQ ID NO:16; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:17, (e) a HCDR2 of SEQ ID NO:18, and (f) a HCDR3 of SEQ ID NO:19;

(vi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:28, (b) a LCDR2 of SEQ ID NO:29, (c) a LCDR3 of SEQ ID NO:30; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:31, (e) a HCDR2 of SEQ ID NO:32, and (f) a HCDR3 of SEQ ID NO:33;

(vii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:42, (b) a LCDR2 of SEQ ID NO:43, (c) a LCDR3 of SEQ ID NO:44; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:45, (e) a HCDR2 of SEQ ID NO:46, and (f) a HCDR3 of SEQ ID NO:47;

(viii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:56, (b) a LCDR2 of SEQ ID NO:57, (c) a LCDR3 of SEQ ID NO:58; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:59, (e) a HCDR2 of SEQ ID NO:60, and (f) a HCDR3 of SEQ ID NO:61;

(ix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:70, (b) a LCDR2 of SEQ ID NO:71, (c) a LCDR3 of SEQ ID NO:72; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:73, (e) a HCDR2 of SEQ ID NO:74, and (f) a HCDR3 of SEQ ID NO:75;

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(x) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:84, (b) a LCDR2 of SEQ ID NO:85, (c) a LCDR3 of SEQ ID NO:86; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:87, (e) a HCDR2 of SEQ ID NO: 88, and (f) a HCDR3 of SEQ ID NO: 89;

(xi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:98, (b) a LCDR2 of SEQ ID NO:99, (c) a LCDR3 of SEQ ID NO:100; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:101, (e) a HCDR2 of SEQ ID NO:102, and (f) a HCDR3 of SEQ ID NO:103;

(xii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:112, (b) a LCDR2 of SEQ ID NO:113, (c) a LCDR3 of SEQ ID NO:114; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:115, (e) a HCDR2 of SEQ ID NO:116, and (f) a HCDR3 of SEQ ID NO:117;

(xiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:126, (b) a LCDR2 of SEQ ID NO:127, (c) a LCDR3 of SEQ ID NO:128; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:129, (e) a HCDR2 of SEQ ID NO:130, and (f) a HCDR3 of SEQ ID NO:131;

(xiv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:140, (b) a LCDR2 of SEQ ID NO:141, (c) a LCDR3 of SEQ ID NO:142; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:143, (e) a HCDR2 of SEQ ID NO:144, and (f) a HCDR3 of SEQ ID NO:145;

(xv) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:154, (b) a LCDR2 of SEQ ID NO:155, (c) a LCDR3 of SEQ ID NO:156; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:157, (e) a HCDR2 of SEQ ID NO:158, and (f) a HCDR3 of SEQ ID NO:159;

(xvi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:168, (b) a LCDR2 of SEQ ID NO:169, (c) a LCDR3 of SEQ ID NO:170; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:171, (e) a HCDR2 of SEQ ID NO:172, and (f) a HCDR3 of SEQ ID NO:173;

(xvii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:182, (b) a LCDR2 of SEQ ID NO:183, (c) a LCDR3 of SEQ ID NO:184; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:185, (e) a HCDR2 of SEQ ID NO:186, and (f) a HCDR3 of SEQ ID NO:187;

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(xviii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:196, (b) a LCDR2 of SEQ ID NO:197, (c) a LCDR3 of SEQ ID NO:198; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:199, (e) a HCDR2 of SEQ ID NO:200, and (f) a HCDR3 of SEQ ID NO:201;

(xix) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:238, (b) a LCDR2 of SEQ ID NO:239, (c) a LCDR3 of SEQ ID NO:240; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:241, (e) a HCDR2 of SEQ ID NO:242, and (f) a HCDR3 of SEQ ID NO:243;

(xx) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:252, (b) a LCDR2 of SEQ ID NO:253, (c) a LCDR3 of SEQ ID NO:254; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:255, (e) a HCDR2 of SEQ ID NO:256, and (f) a HCDR3 of SEQ ID NO:257;

(xxi) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:280, (b) a LCDR2 of SEQ ID NO:281, (c) a LCDR3 of SEQ ID NO:282; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:283, (e) a HCDR2 of SEQ ID NO:284, and (f) a HCDR3 of SEQ ID NO:285;

(xxii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:294, (b) a LCDR2 of SEQ ID NO:295, (c) a LCDR3 of SEQ ID NO:296; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:297, (e) a HCDR2 of SEQ ID NO:298, and (f) a HCDR3 of SEQ ID NO:299; or

(xxiii) a light chain variable region that comprises (a) a LCDR1 (CDR-Complementarity Determining Region) of SEQ ID NO:322, (b) a LCDR2 of SEQ ID NO:323, (c) a LCDR3 of SEQ ID NO:324; and a heavy chain variable region that comprises: (d) a HCDR1 of SEQ ID NO:325, (e) a HCDR2 of SEQ ID NO:326, and (f) a HCDR3 of SEQ ID NO:327.

58. The antibody or antigen binding fragment thereof of claim 57 in which at least one amino acid within a CDR is substituted by a corresponding residue of a corresponding CDR of another anti-CDH6 antibody in Table 5 or 6.

59. The antibody or antigen binding fragment thereof of claim 57, wherein said antibody or antigen binding fragment thereof comprises:

(i) a heavy chain variable region (vH) that comprises SEQ ID NO: 230, and a light chain variable region (vL) that comprises SEQ ID NO:231;

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(ii) a heavy chain variable region (vH) that comprises SEQ ID NO: 216, and a light chain variable region (vL) that comprises SEQ ID NO:217;

(iii) a heavy chain variable region (vH) that comprises SEQ ID NO: 272, and a light chain variable region (vL) that comprises SEQ ID NO:273;

(iv) a heavy chain variable region (vH) that comprises SEQ ID NO:314, and a light chain variable region (vL) that comprises SEQ ID NO:315;

(v) a heavy chain variable region (vH) that comprises SEQ ID NO:20, and a light chain variable region (vL) that comprises SEQ ID NO:21;

(vi) a heavy chain variable region (vH) that comprises SEQ ID NO: 34, and a light chain variable region (vL) that comprises SEQ ID NO:35;

(vii) a heavy chain variable region (vH) that comprises SEQ ID NO:48, and a light chain variable region (vL) that comprises SEQ ID NO:49;

(viii) a heavy chain variable region (vH) that comprises SEQ ID NO:62, and a light chain variable region (vL) that comprises SEQ ID NO:63;

(ix) a heavy chain variable region (vH) that comprises SEQ ID NO:76, and a light chain variable region (vL) that comprises SEQ ID NO:77;

(x) a heavy chain variable region (vH) that comprises SEQ ID NO:90, and a light chain variable region (vL) that comprises SEQ ID NO:91;

(xi) a heavy chain variable region (vH) that comprises SEQ ID NO:104, and a light chain variable region (vL) that comprises SEQ ID NO:105;

(xii) a heavy chain variable region (vH) that comprises SEQ ID NO: 118, and a light chain variable region (vL) that comprises SEQ ID NO:119;

(xiii) a heavy chain variable region (vH) that comprises SEQ ID NO: 132, and a light chain variable region (vL) that comprises SEQ ID NO:133;

(xiv) a heavy chain variable region (vH) that comprises SEQ ID NO: 146, and a light chain variable region (vL) that comprises SEQ ID NO:147;

(xv) a heavy chain variable region (vH) that comprises SEQ ID NO:160, and a light chain variable region (vL) that comprises SEQ ID NO:161;

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(xvi) a heavy chain variable region (vH) that comprises SEQ ID NO: 174, and a light chain variable region (vL) that comprises SEQ ID NO:175;

(xvii) a heavy chain variable region (vH) that comprises SEQ ID NO: 188, and a light chain variable region (vL) that comprises SEQ ID NO:189;

(xviii) a heavy chain variable region (vH) that comprises SEQ ID NO: 202, and a light chain variable region (vL) that comprises SEQ ID NO:203;

(xix) a heavy chain variable region (vH) that comprises SEQ ID NO: 244, and a light chain variable region (vL) that comprises SEQ ID NO:245;

(xx) a heavy chain variable region (vH) that comprises SEQ ID NO:258, and a light chain variable region (vL) that comprises SEQ ID NO:259;

(xxi) a heavy chain variable region (vH) that comprises SEQ ID NO:286, and a light chain variable region (vL) that comprises SEQ ID NO:287;

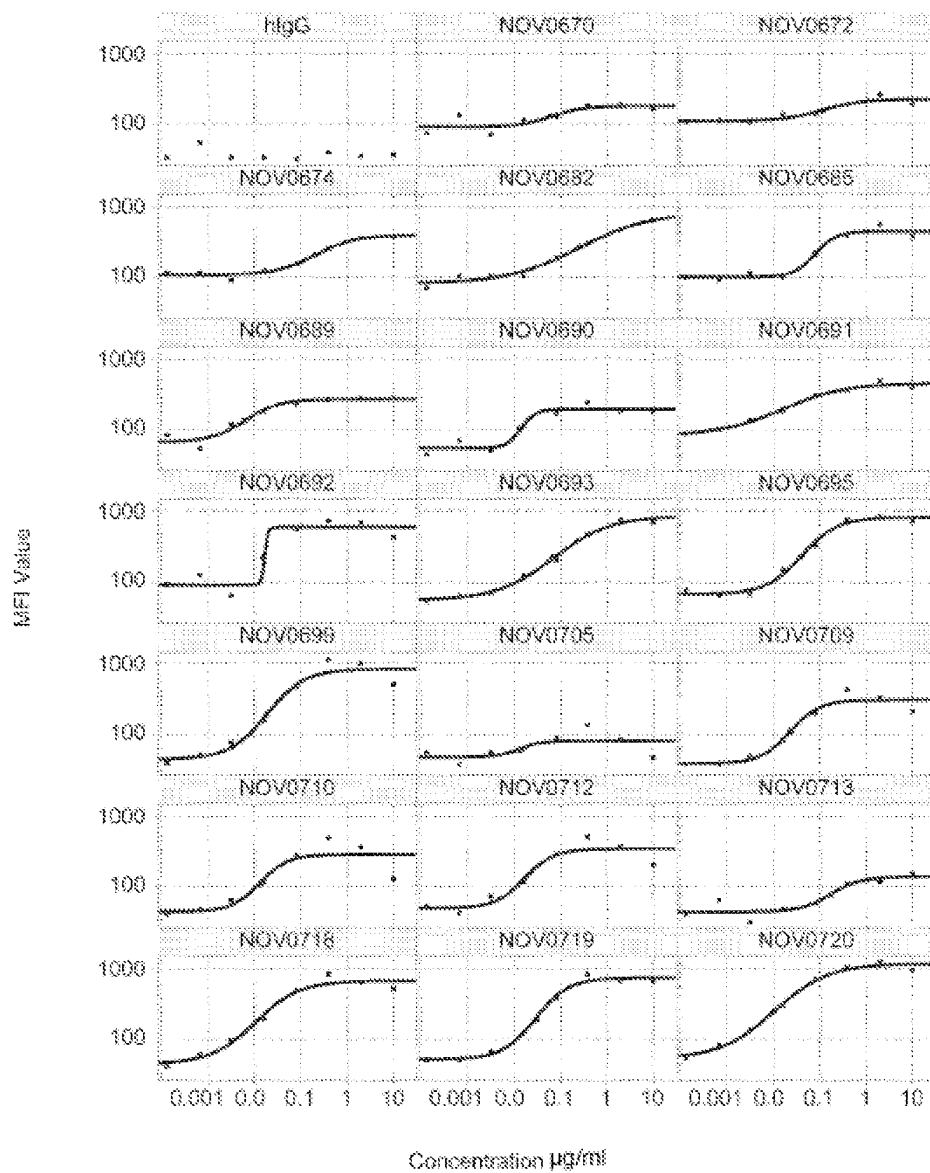
(xxii) a heavy chain variable region (vH) that comprises SEQ ID NO:300, and a light chain variable region (vL) that comprises SEQ ID NO:301; or

(xxiii) a heavy chain variable region (vH) that comprises SEQ ID NO:328, and a light chain variable region (vL) that comprises SEQ ID NO:329.

60. A diagnostic reagent comprising the antibody or antigen binding fragment thereof of claim 57 or 59 which is labeled.

61. The diagnostic reagent of claim 60, wherein the label is selected from the group consisting of a radiolabel, a fluorophore, a chromophore, an imaging agent, and a metal ion.

Determination of cellular binding EC50 of CDH6 IgGs by FACS on OVCAR3 cells



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FIGURE 2

Epitope binning of anti-CDH6 antibodies using competitive binding assay on Biacore

	NOV1126	NOV1127	NOV1132	NOV0692	NOV0710	NOV0712	NOV0719
NOV1126	8.59	16.47	23.7	7.829	15	9.506	25.42
NOV1127	56.62	36.74	39.28	50.79	32.73	24.73	46.9
NOV1132	30.06	17.82	1.08	23.2	10.62	6.004	20.71
NOV0692	18.7	25.45	17.87	-2.84	8.84	5.12	13.48
NOV0710	36.93	30.92	17.84	17.89	-3.562	-3.264	16.15
NOV0712	26.66	22.38	14.3	14.14	-2.167	-1.821	11.05
NOV0719	61.59	51.5	33.65	35.53	21.94	16.48	-2.356

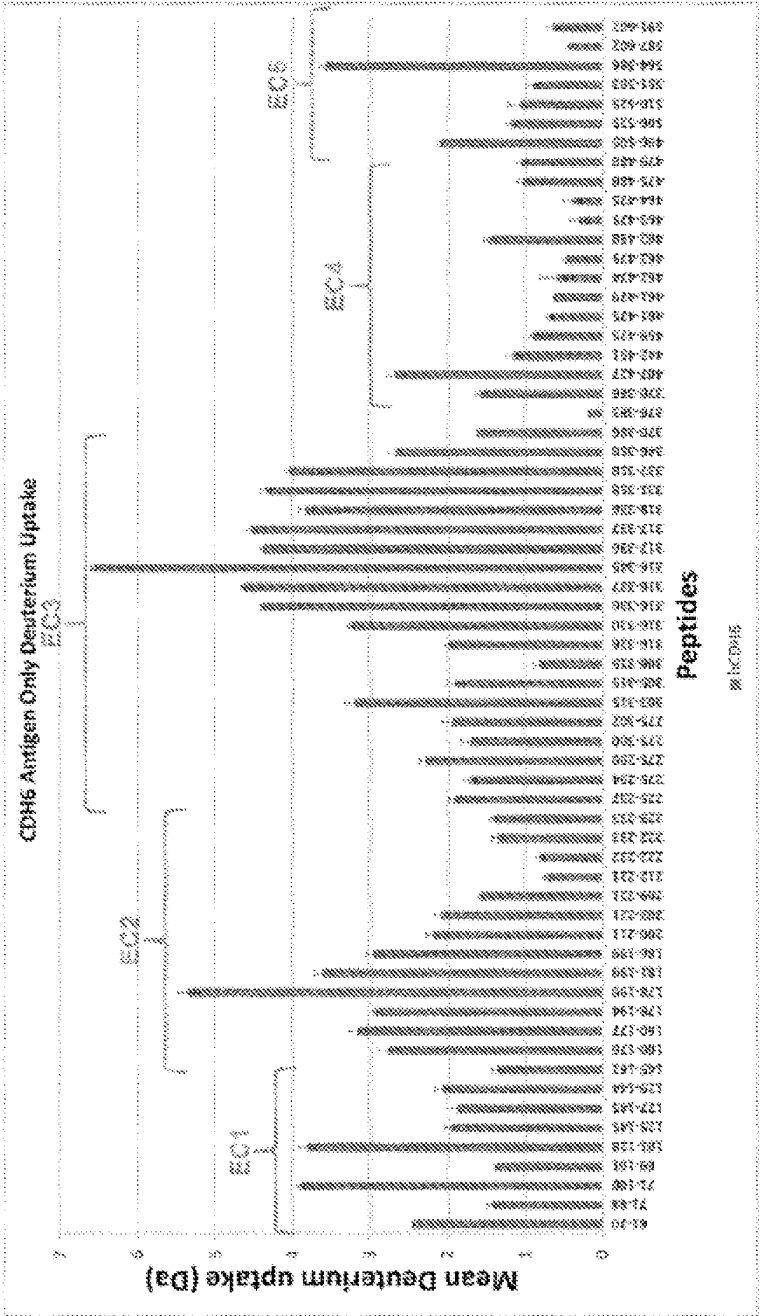
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FIGURE 3

hCDH6(34-615) ion-exchange data summary



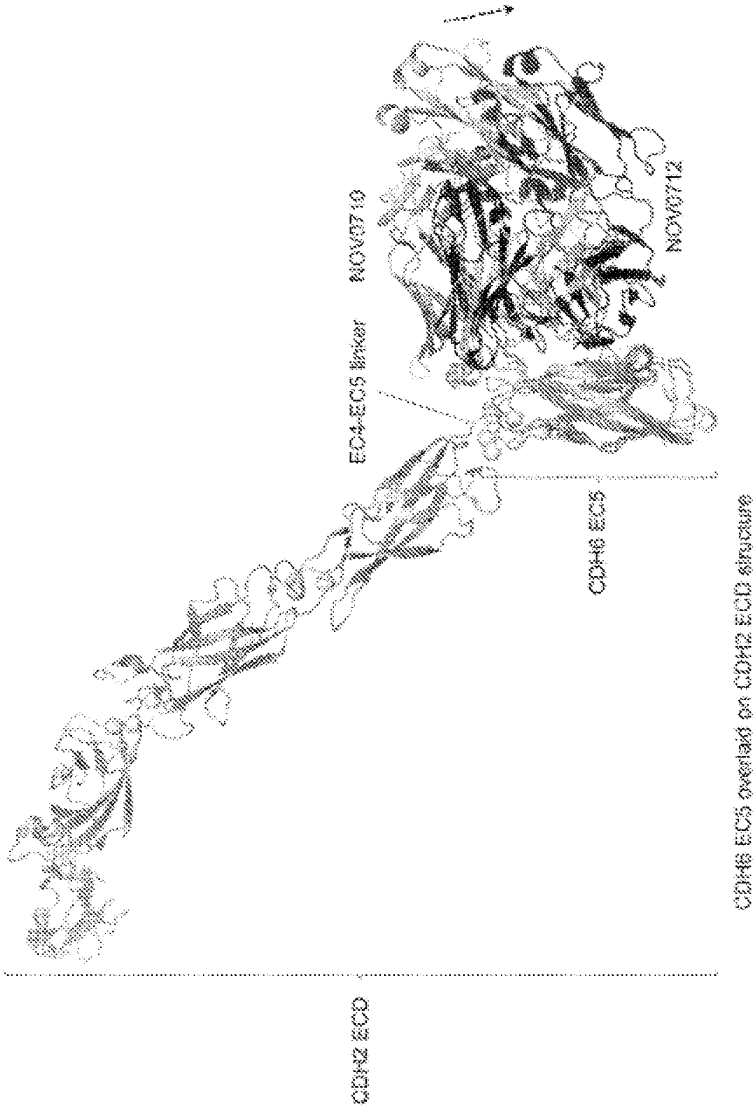
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FIGURE 4

Overlay of CDH6 ECS/NOV0712 and CDH6 ECS/NOV0710 complex structures onto the full-length ECD structure of CDH2 by superposition of ECS domains. The lighter-colored ribbon is NOV0710, the darker one is NOV0712



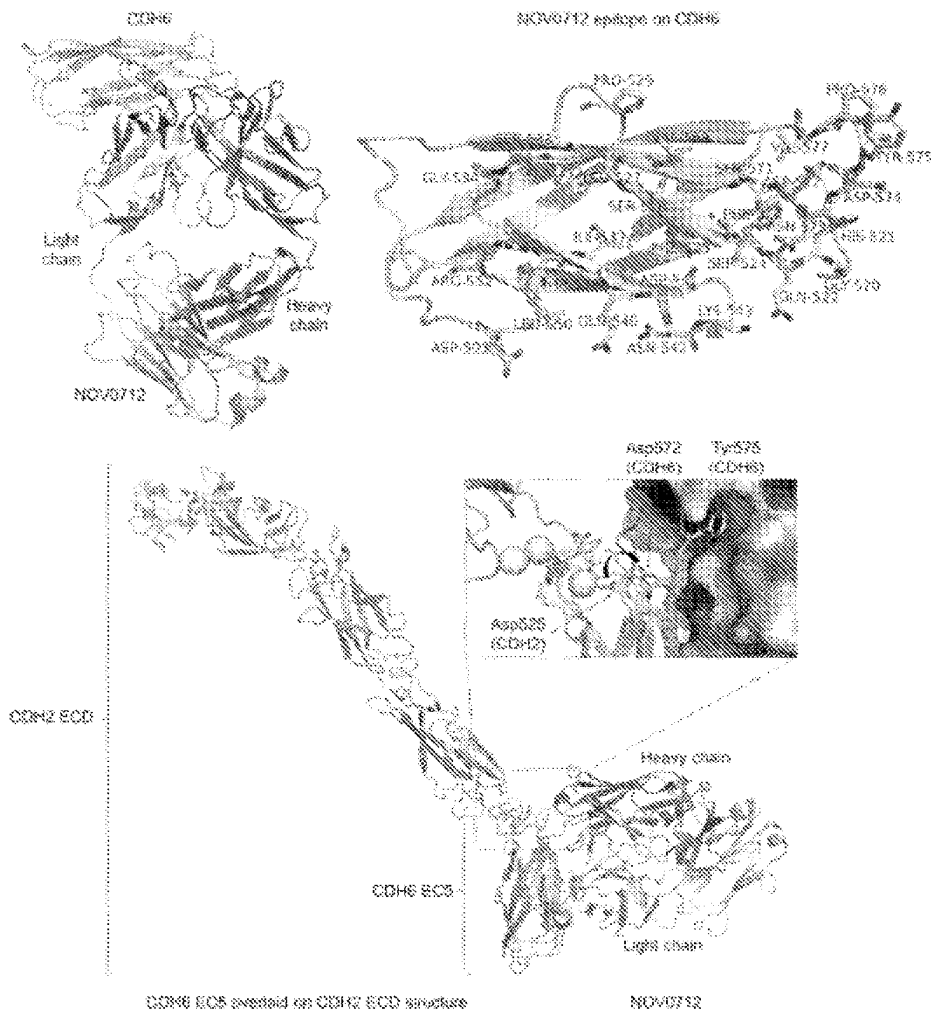
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FIGURE 5

Upper panel: Structure of NOV0712 binding to CDH6. Upper panel: overall structure of NOV0712 binding to CDH6 EC5 (left) and detailed view of NOV0712 epitope residues on CDH6 (right, shown as sticks and labeled). Lower panel: NOV0712 binds the majority of a Ca^{2+} -binding loop in CDH6 EC5 (residues 571-579) and induces an "out" conformation of the loop



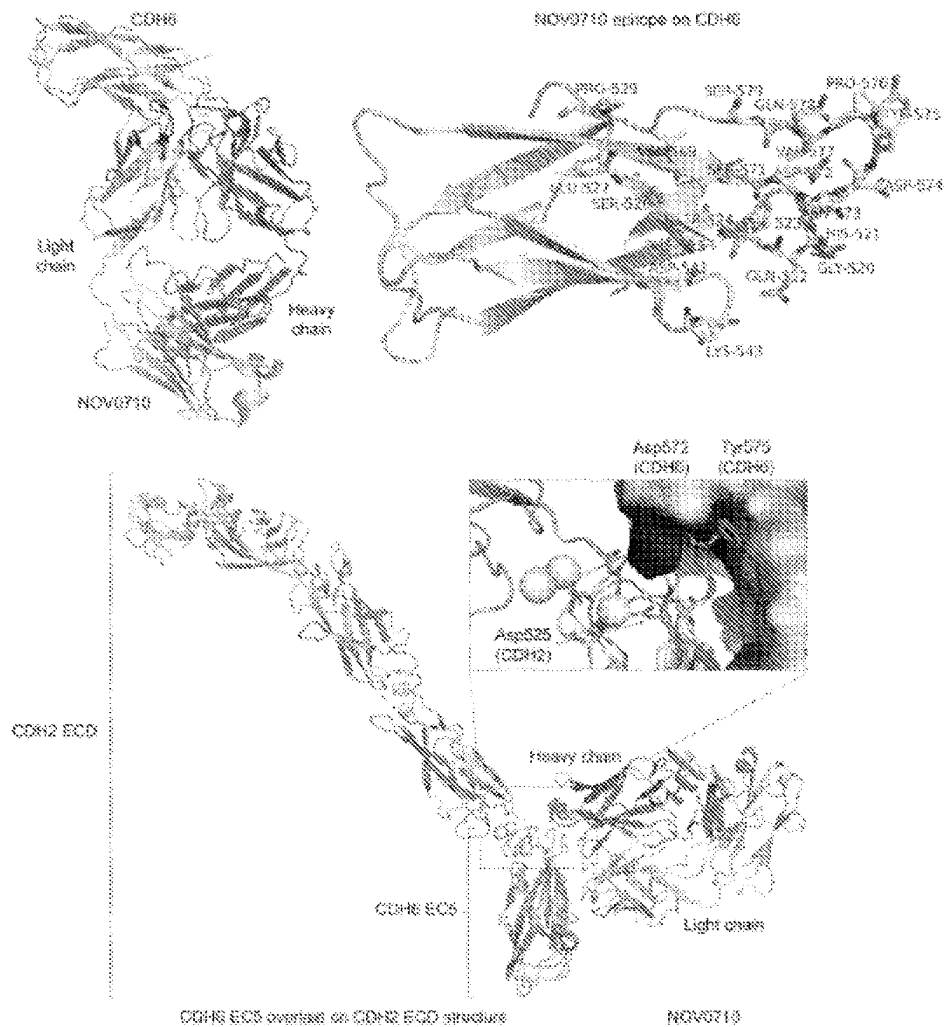
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FIGURE 6

Upper panel: Structure of NOV0710 binding to CDH6. Upper panel: overall structure of NOV0710 binding to CDH6 EC5 (left) and detailed view of NOV0710 epitope residues on CDH6 (right, shown as sticks and labeled). Lower panel: NOV0710 binds the majority of a Ca^{2+} -binding loop in CDH6 EC5 (residues 571-579) and induces an "out" conformation of the loop



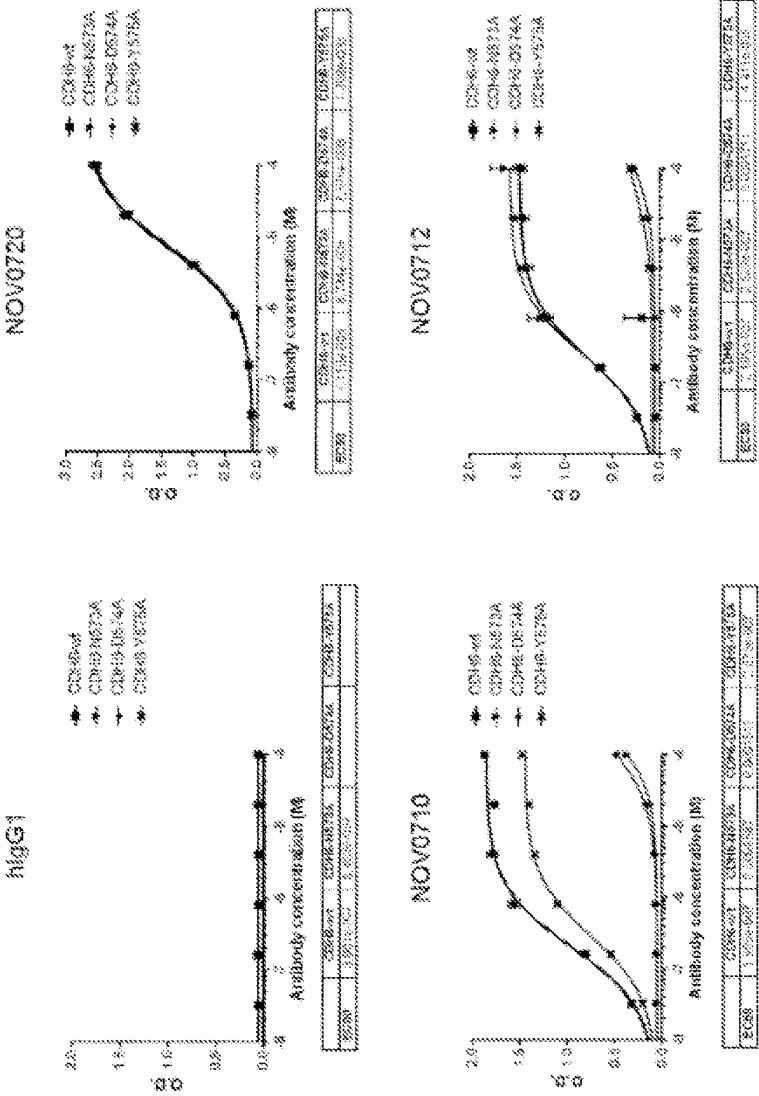
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FIGURE 7

ELISA assay to evaluate binding of CDH6 antibodies to wild-type and mutant forms of CDH6



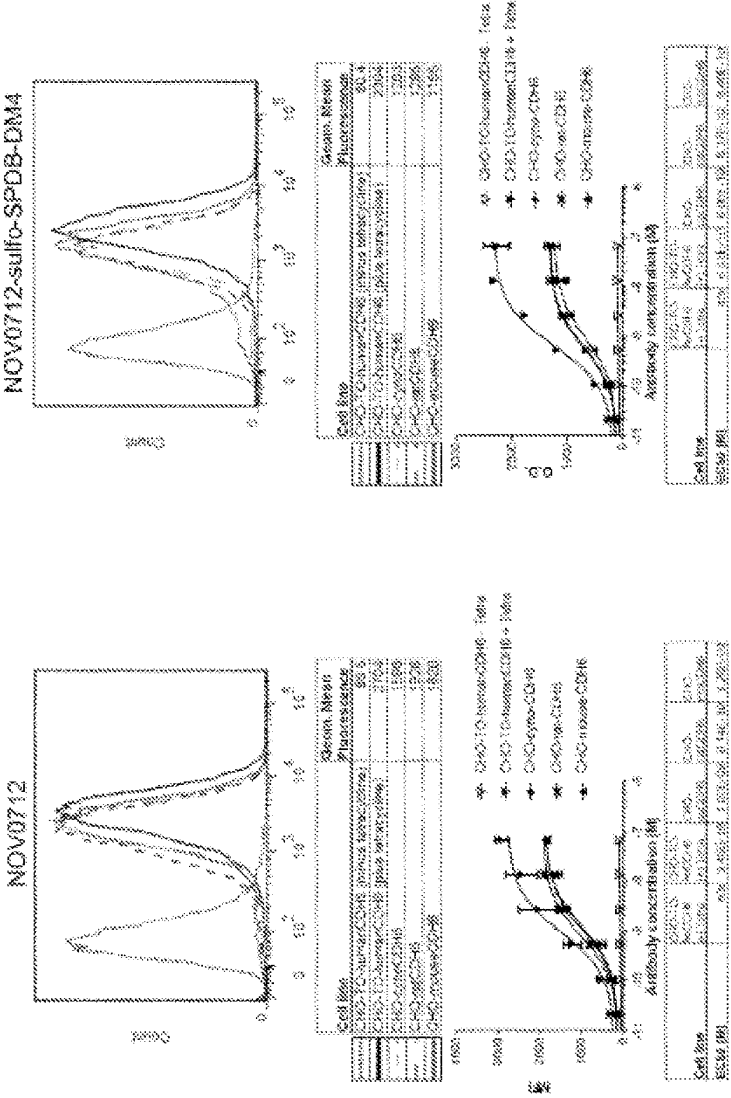
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FIGURE 8

Cellular binding of of CDH6-targeting antibodies in either non-conjugated form or as a sulfo-SPDB-DM4 antibody drug conjugate in a panel of CHO cell lines expressing either human, cynomolgus, rat or mouse CDH6



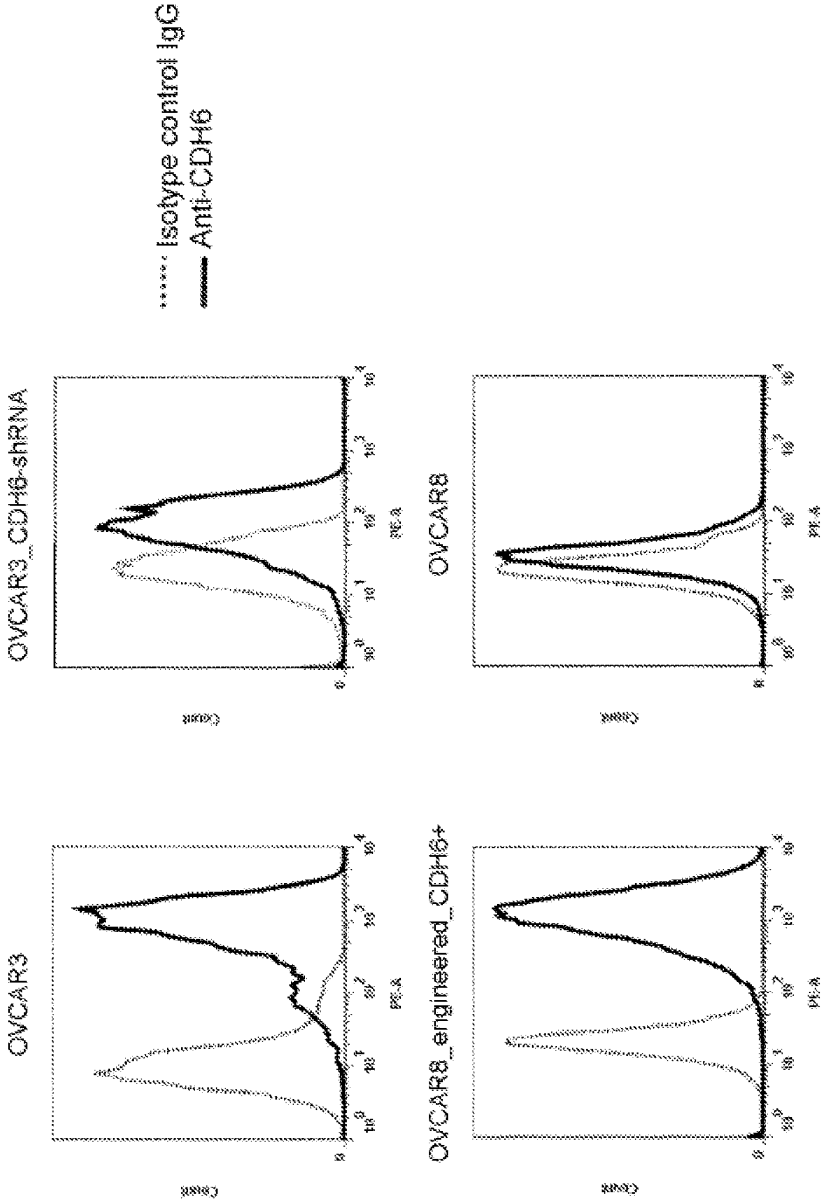
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FIGURE 9

FACS analysis of CDH6 expression in ovarian cancer cell lines OVCAR3, OVCAR3_CDH6_shRNA, OVCAR8 and OVCAR8_engineered_CDH6+



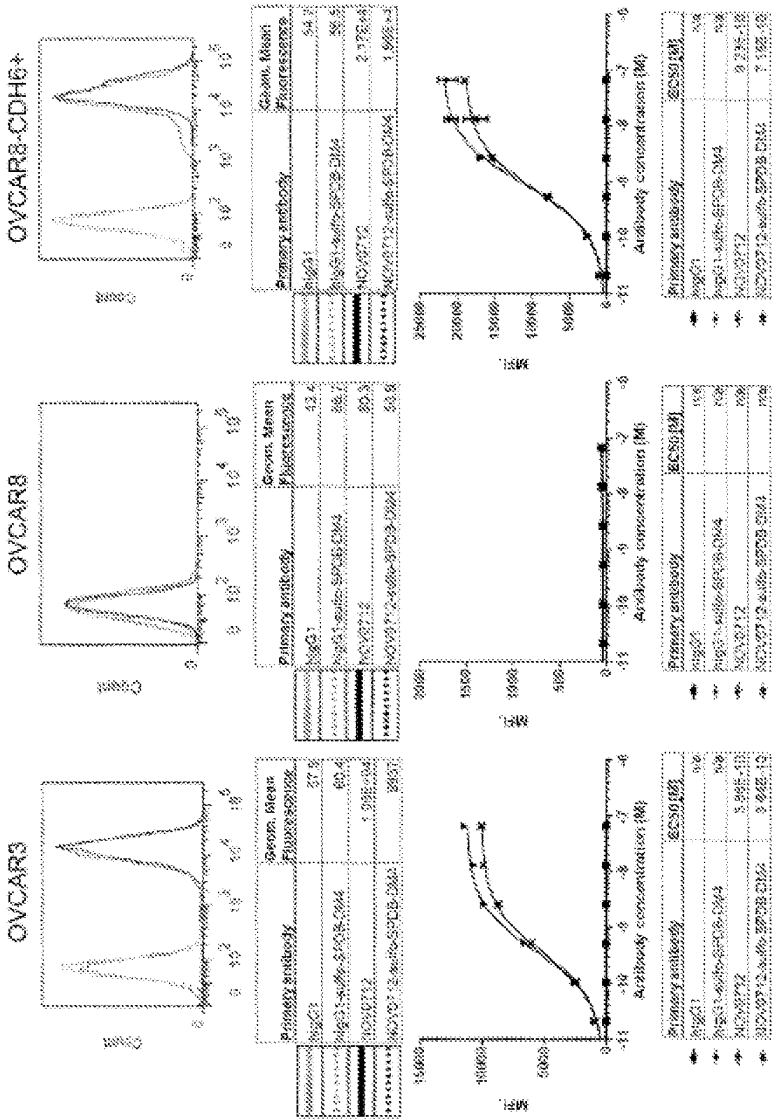
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FIGURE 10

Cellular binding of of CDH16-targeting antibodies or control IgG in either non-conjugated form or as a sulfo-SPDB-DM4 antibody drug conjugate in a panel of ovarian cancer cell lines



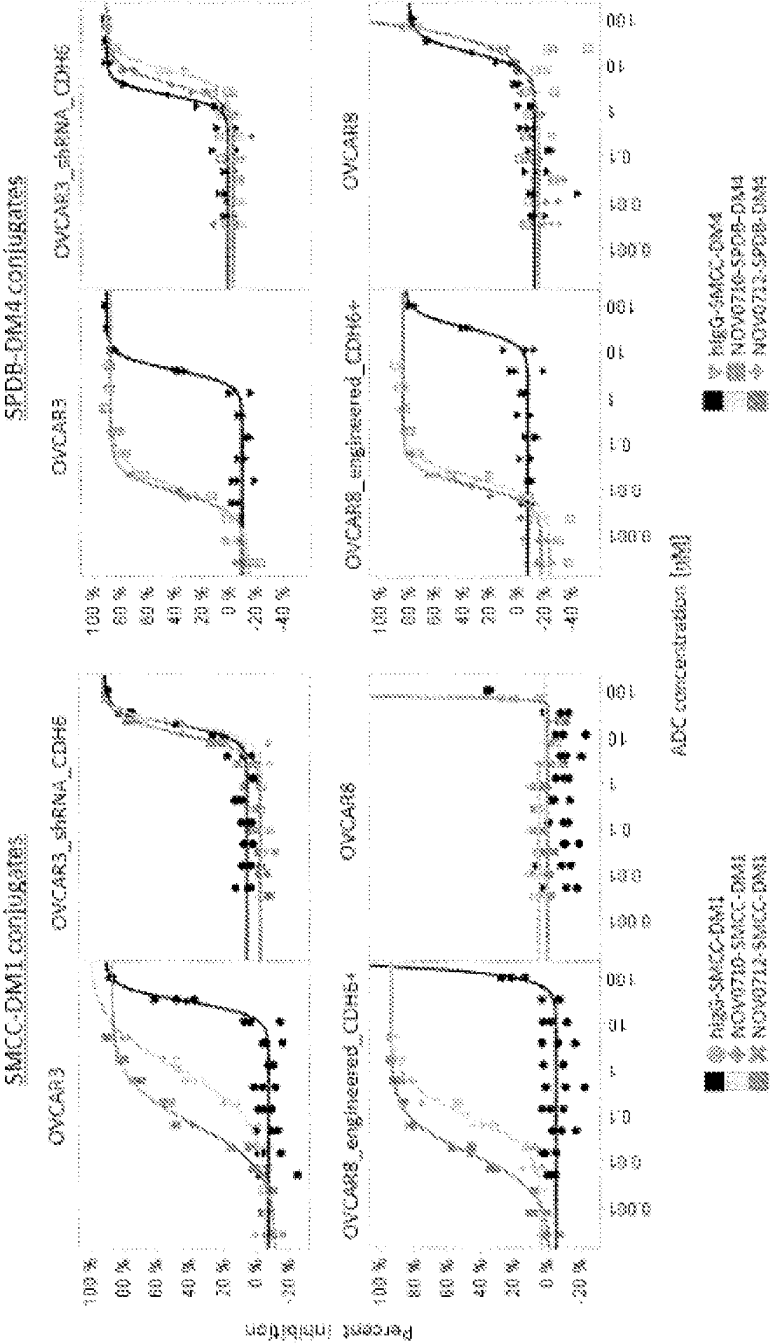
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FIGURE 11

In vitro cellular activity of anti-CDH6 ADCs as SMCC-DM1 and SPDB-DM4 conjugates on ovarian cancer cell lines



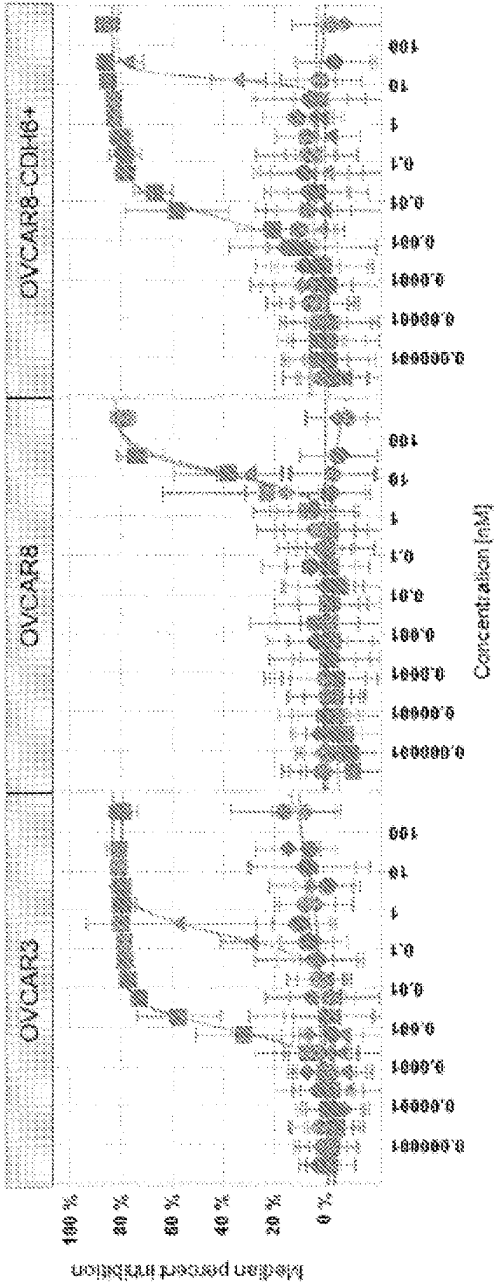
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FIGURE 12

In vitro cellular activity of CDH6-targeting antibodies or control IgG in either non-conjugated form or as a sulfo-SPDB-DM4 antibody drug conjugate



Legend:

- NOV0712-sulfo-SPDB-DM4
- NOV0712
- hIgG1
- hIgG1-sulfo-SPDB-DM4

IC50 [nM]			
Treatment	OVCAR3	OVCAR8	OVCAR8-CDH6+
NOV0712-sulfo-SPDB-DM4	1.28E-03	1.28E+01	5.28E-03
NOV0712	>333	>333	>333
hIgG1	>333	>333	>333
hIgG1-sulfo-SPDB-DM4	3.34E-01	1.28E+01	1.48E+0

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FIGURE 13

In vivo efficacy of anti-CDH6 antibodies as SMCC-DM1 conjugates in a xenograft mouse model of ovarian cancer

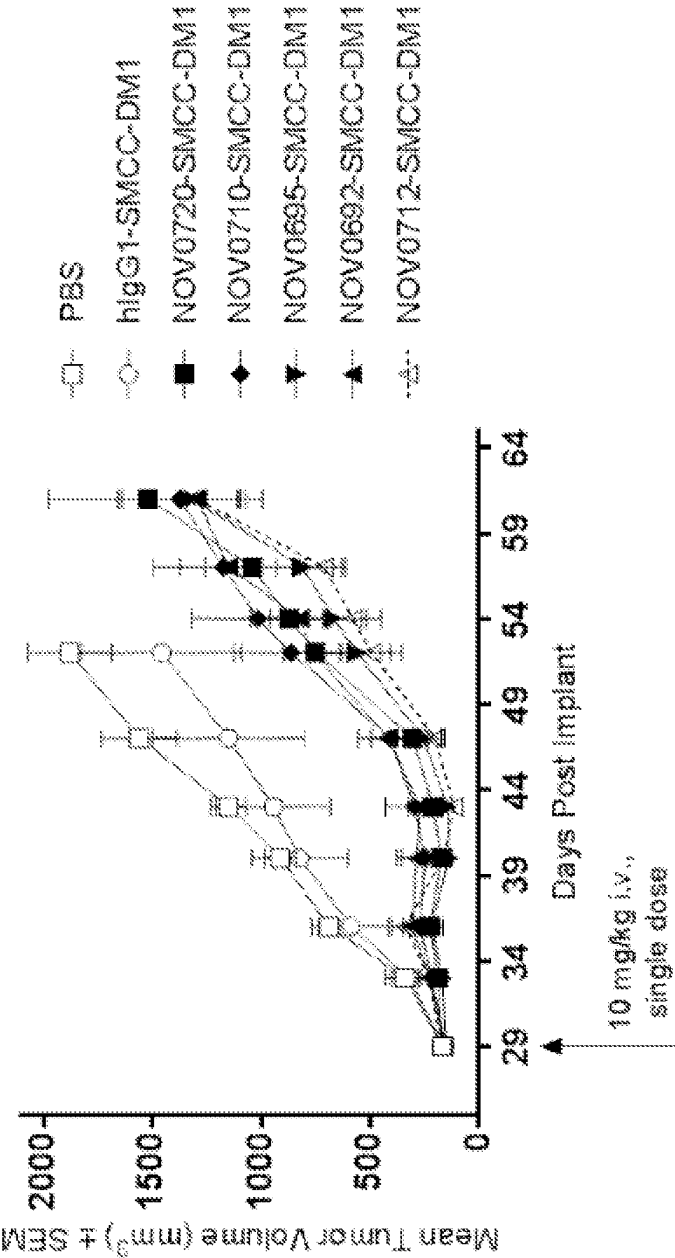
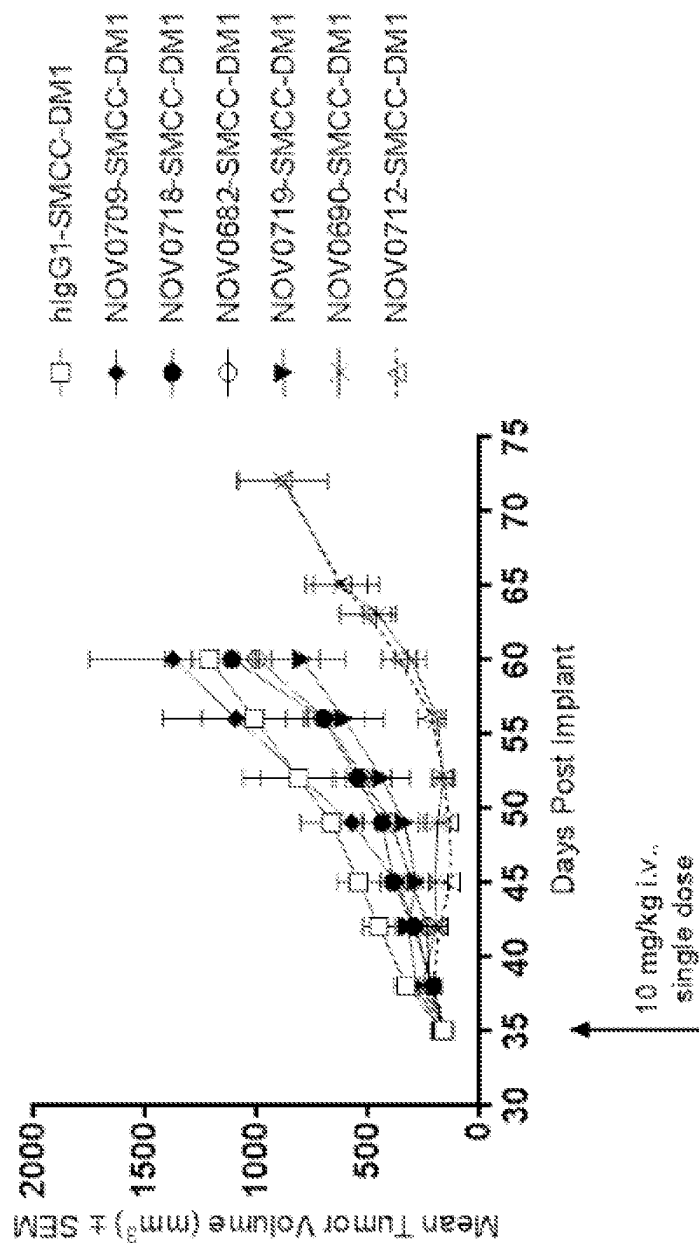


FIGURE 14

In vivo efficacy of anti-CD46 antibodies as SMCC-DM1 conjugates in a xenograft mouse model of ovarian cancer



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FIGURE 16

In vivo efficacy of anti-CTDE6 antibodies as SPDB-DM4 and SMCC-DM1 conjugates in a xenograft mouse model of ovarian cancer

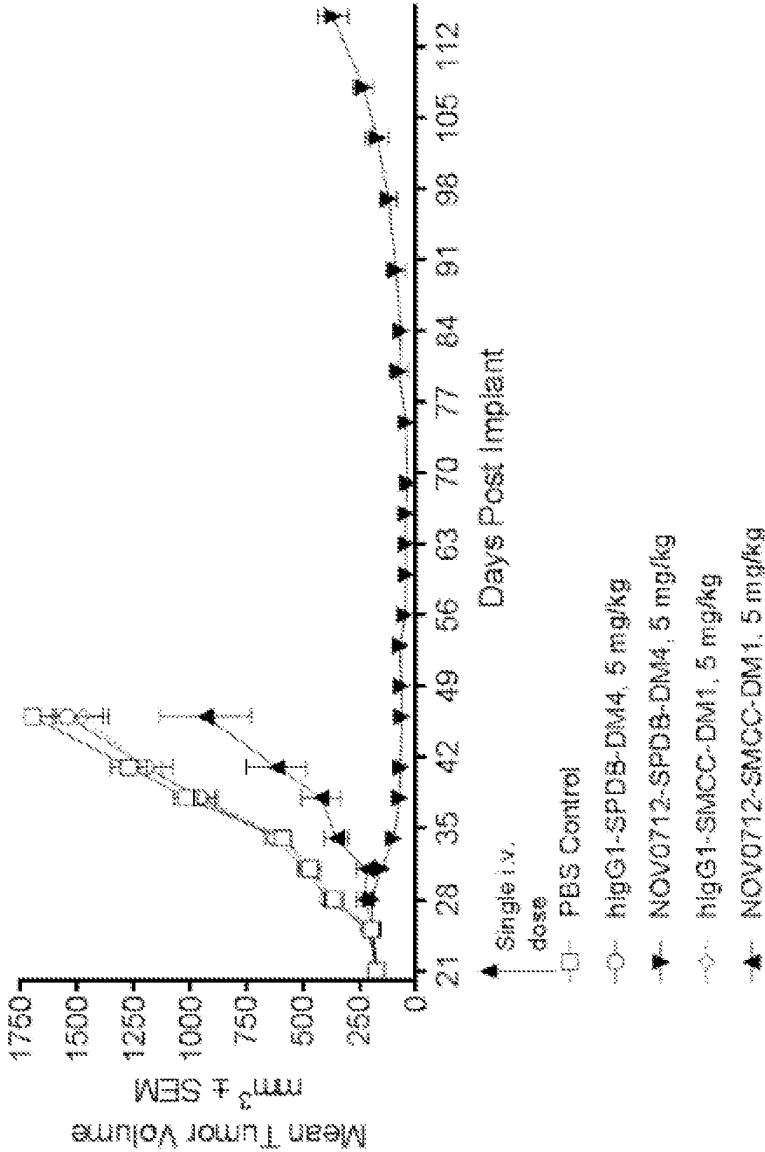
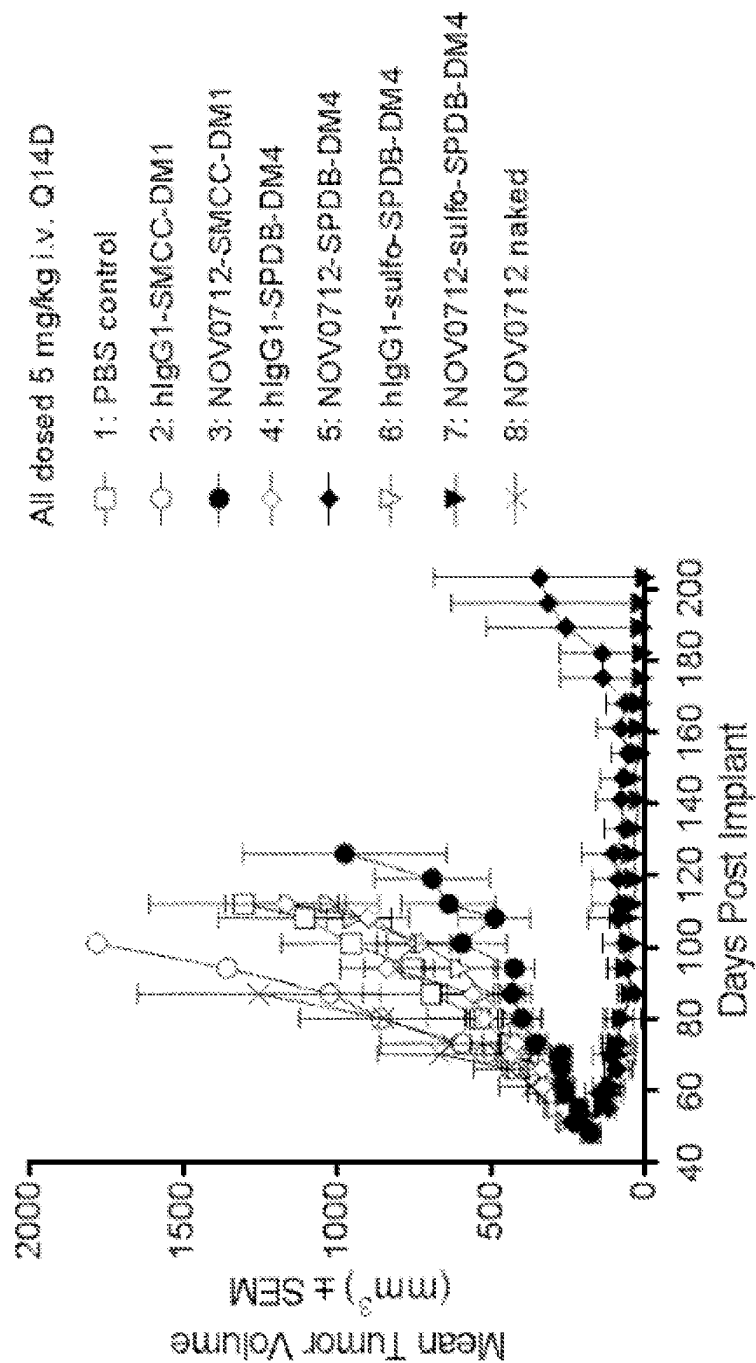


FIGURE 17

In vivo efficacy of anti-CTH46 ADCs with different linker/payload formats in a patient-derived primary tumor xenograft mouse model of ovarian cancer (serous carcinoma)



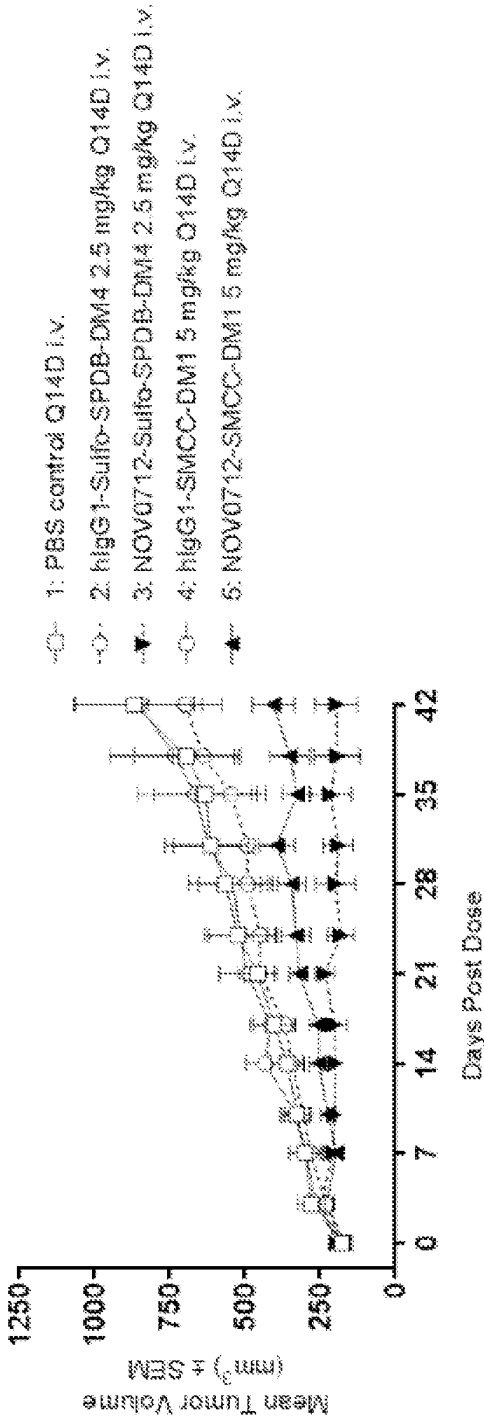
WO 2016/024195

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PCT/IB2015/056032

FIGURE 19

In vivo efficacy of anti-CTEB6 ADCs with different linker/payload formats in a patient-derived primary tumor xenograft mouse model of ovarian cancer (serous carcinoma)



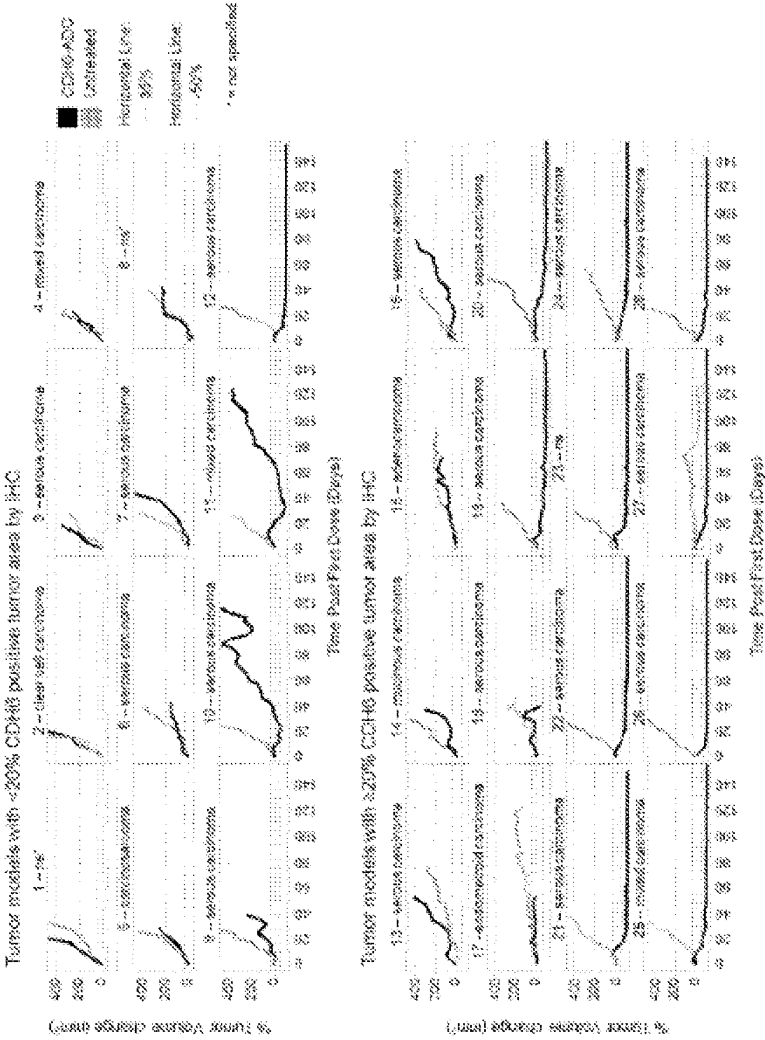
WO 2016/024195

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PCT/IB2015/056032

FIGURE 20

In vivo efficacy of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4, 5 mg/kg Q14D, i.v.) in a panel of patient-derived primary tumor xenograft mouse models of ovarian cancer



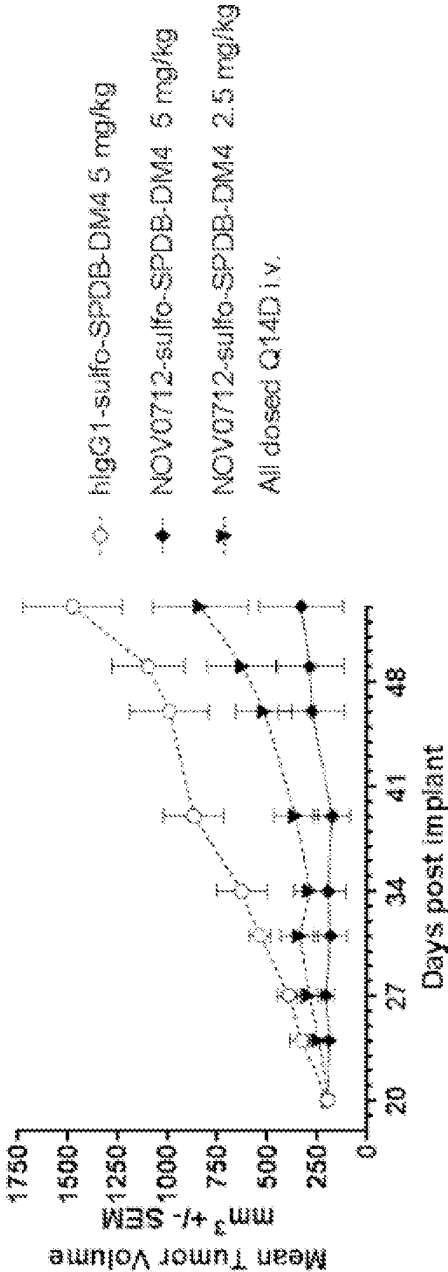
WO 2016/024195

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PCT/IB2015/056032

FIGURE 21

In vivo efficacy of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4), dosed either at 2.5 mg/kg, Q14D or 5 mg/kg, Q14D in a patient-derived primary tumor xenograft mouse model of renal cancer (renal cell carcinoma)



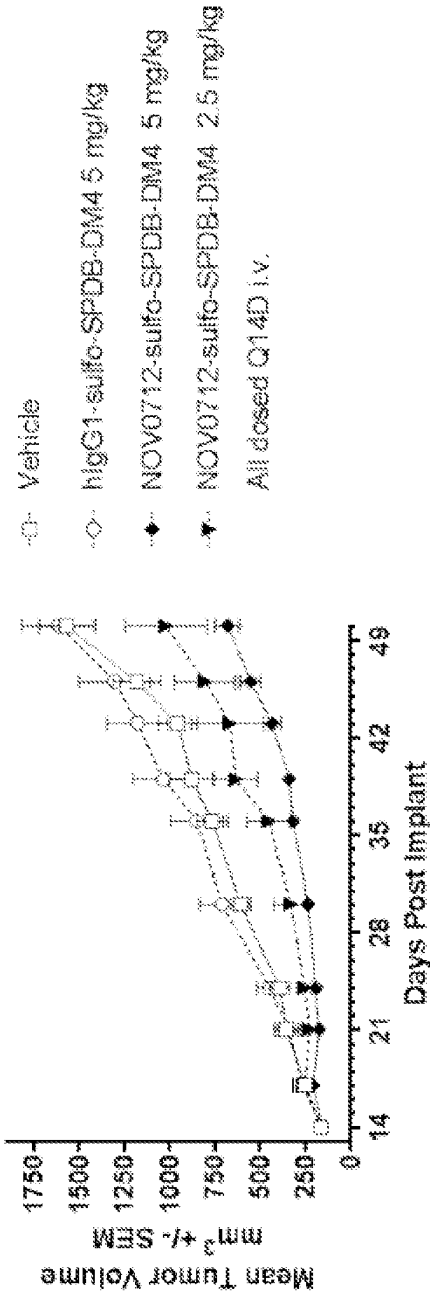
WO 2016/024195

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PCT/IB2015/056032

FIGURE 22

In vivo efficacy of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4), dosed either at 2.5 mg/kg, Q14D or 5 mg/kg, Q14D in a patient-derived primary tumor xenograft mouse model of renal cancer (metastatic lesion of renal cell carcinoma, clear cell type)



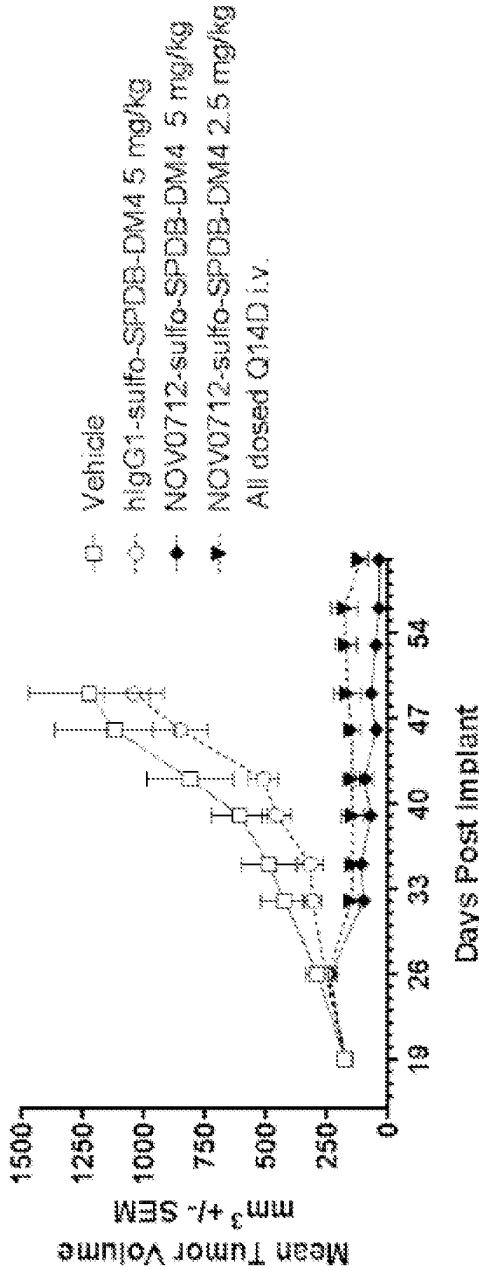
WO 2016/024195

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FIGURE 23

In vivo efficacy of an anti-CDH6 ADC (NOV0712-sulfo-SPDB-DM4), dosed either at 2.5 mg/kg, Q14D or 5 mg/kg, Q14D in a patient-derived primary tumor xenograft mouse model of renal cancer (renal cell carcinoma, clear cell type)



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FIGURE 24

Evidence for combination activity of CDH6-4targeting ADCs with BCL2/BCL-XL, BCL-XL, IAP and MEK inhibitors

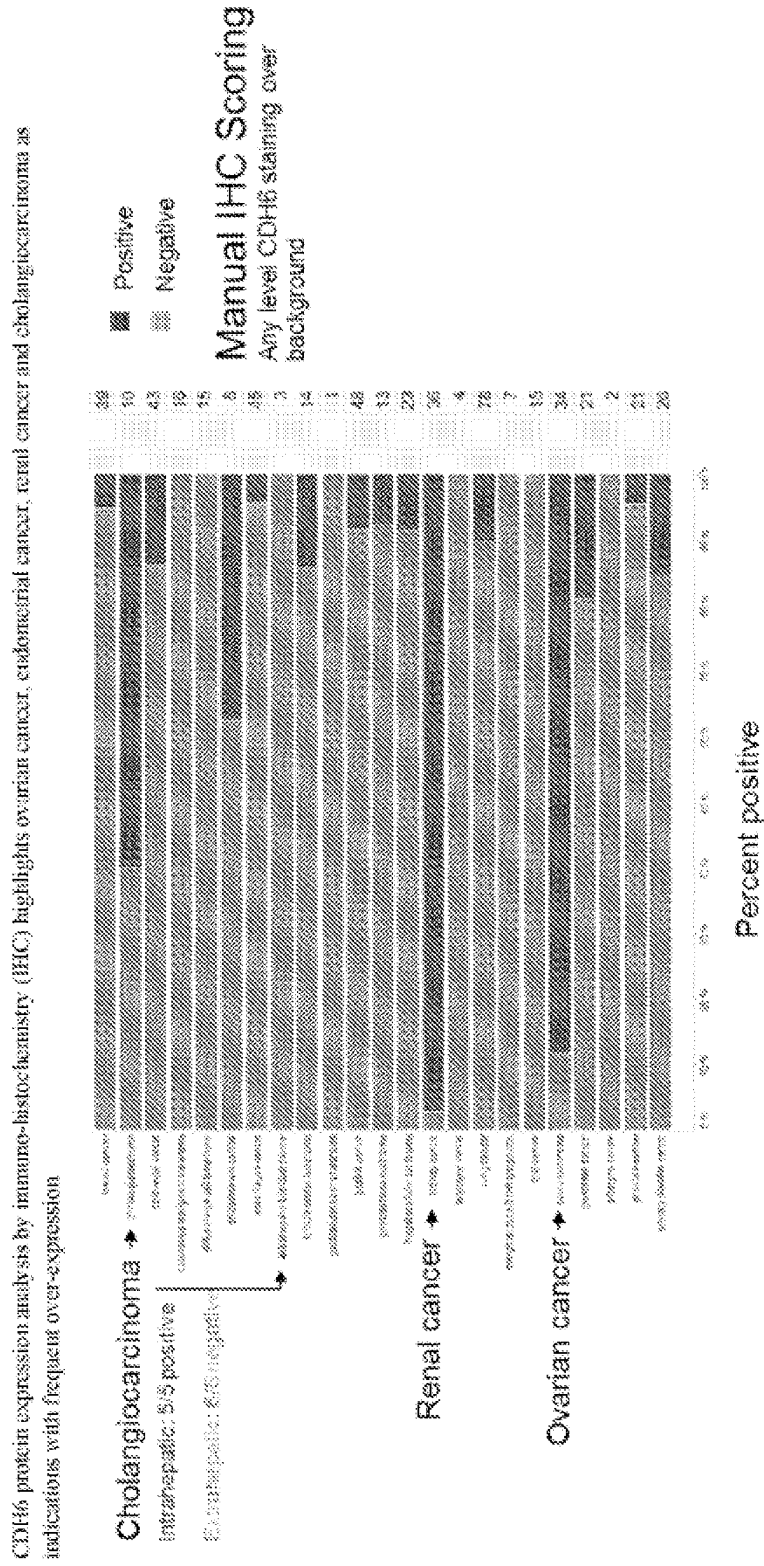
Combination	Loewe Synergy Score	Loewe Excess Average
CDH6-ADC + CDH6-ADC	7.79	0.078
ABT-263 + ABT-263	6.04	0.055
WEHI-539 + WEHI-539	3.30	0.079
NVP-LCL161 + NVP-LCL161	0.0	-0.660
CDH6-ADC + ABT-263	41	0.364
CDH6-ADC + WEHI-539	31.3	0.316
CDH6-ADC + NVP-LCL161	17.9	0.187
CDH6-ADC + irametinib	26.9	0.258

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FIGURE 26



INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2015/056032

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K16/28 A61K39/395 A61P35/00 A61K31/00 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C07K G01N A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPD-Internal, WPI Data, BIOSIS, EMBASE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim(s)
Y	WO 2005/001092 A2 (WYETH CORP [US]; BE XIAOBING [US]; WEI LIU [US]; SLONIM DONNA K [US];) 6 January 2005 (2005-01-06) claims 1,4 example 1 ----- -/--	1-4,7, 10-24, 27, 29-50, 55,56
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "Z" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
10 December 2015		12/01/2016
Name and mailing address of the ISA/ European Patent Office, P.O. 5818 Patentlaan 2 NL - 2230 HV Rijswijk Tel: (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Bumb, Peter

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2015/056032

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHIMAZUI T ET AL: "Expression of cadherin-6 as a novel diagnostic tool to predict prognosis of patients with E-cadherin-absent renal cell carcinoma.", CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH OCT 1998, vol. 4, no. 10, October 1998 (1998-10), pages 2419-2424, XP002750983, ISSN: 1078-0432 page 2420, left-hand column, line 8 - right-hand column, line 9 -----	5,6,25, 26, 51-54, 57,58, 60,61
Y	PAUL ET AL: "Cadherin-6:: A New Prognostic Marker for Renal Cell Carcinoma", JOURNAL OF UROLOGY, LIPPINCOTT WILLIAMS & WILKINS, BALTIMORE, MD, US, vol. 171, no. 1, 1 January 2004 (2004-01-01), pages 97-101, XP005532199, ISSN: 0022-5347, DOI: 10.1097/01.JU.0000101512.47242.79 page 97, right-hand column, line 8 - line 21 page 100, right-hand column, last paragraph -----	1-4,7, 10-24, 27, 29-50, 55,56
Y	ROBERT Y. ZHAO ET AL: "Synthesis and Evaluation of Hydrophilic Linkers for Antibody-Maytansinoid Conjugates", JOURNAL OF MEDICINAL CHEMISTRY, vol. 54, no. 10, 26 May 2011 (2011-05-26), pages 3606-3623, XP055046274, ISSN: 0022-2623, DOI: 10.1021/jm2002958 page 3612, left-hand column, line 37 page 3612, right-hand column, lines 24, 35-38 page 3615, left-hand column, lines 12-16 -----	22-24, 27,29-36
Y	VALENTINA SANCISI ET AL: "Cadherin 6 Is a New RUNX2 Target in TGF-[beta] Signalling Pathway", PLOS ONE, vol. 8, no. 9, 12 September 2013 (2013-09-12), page e75489, XP55235108, DOI: 10.1371/journal.pone.0075489 the whole document -----	1-4,7, 10-24, 27, 29-50, 55,56

information on past and future members.

PCT/IB2015/056032

From: POC@NSA.gov (external email) (Sent: 2/20/2016)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2018/018572

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12N15/13, A61K9/00, A61K31/4748, A61K39/395, A61K47/68,
A61P13/12, A61P15/08, A61P35/00, A61P43/00, C07K16/28,
C12N1/15, C12N1/19, C12N1/21, C12N5/10, C12P21/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2018
Registered utility model specifications of Japan 1996-2018
Published registered utility model applications of Japan 1994-2018

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

JSTPlus/JMEDPlus/JST7580 (JDreamIII), CAPLUS/MEDLINE/EMBASE/Biosis (STN),
WPIDS/WPIX (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/024195 A1 (NOVARTIS AG) 18 February 2016, claims 1-61, examples, in particular, example 7, fig. 4, etc. & US 2016/0046711 A1, claims 1-61, examples, in particular, example 7, fig. 4, etc.	1-53
A	WO 2014/057697 A1 (DAIICHI SANKYO CO., LTD.) 17 April 2014, claims 1-49, examples, etc. & US 2015/0297748 A1, claims 1-49, examples, etc.	1-53



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 July 2018 (30.07.2018)

Date of mailing of the international search report

07 August 2018 (07.08.2018)

Name and mailing address of the ISA/

Japan Patent Office
3-4-3, Kasumigaseki, Chiyoda-ku,
Tokyo 100-8915, Japan

Authorized officer

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2018/018572

CLASSIFICATION OF SUBJECT MATTER

C12N15/13(2006.01)i, A61K9/00(2006.01)i, A61K31/4745(2006.01)i,
A61K39/395(2006.01)i, A61K47/68(2017.01)i, A61P13/12(2006.01)i,
A61P15/08(2006.01)i, A61P35/00(2006.01)i, A61P43/00(2006.01)i,
C07K16/28(2006.01)i, C12N1/15(2006.01)i, C12N1/19(2006.01)i,
C12N1/21(2006.01)i, C12N5/10(2006.01)i, C12P21/08(2006.01)i

国際調査報告		国際出願番号 PCT/J P 2018/018572	
A. 発明の属する分野の分類 (国際特許分類 (IPC)) Int.Cl. 特別ページ参照			
B. 調査を行った分野 調査を行った最小限資料 (国際特許分類 (IPC)) Int.Cl. C12N15/13, A61K9/00, A61K31/4745, A61K39/395, A61K47/68, A61P13/12, A61P15/08, A61P35/00, A61P43/00, C07K16/28, C12N1/15, C12N1/19, C12N1/21, C12N5/19, C12P21/08 最小限資料以外の資料で調査を行った分野に含まれるもの 日本国実用新案公報 1922-1996年 日本国公開実用新案公報 1971-2018年 日本国実用新案登録公報 1996-2018年 日本国登録実用新案公報 1994-2018年			
国際調査で利用した電子データベース (データベースの名称、調査に使用した用語) JSTPlus/JMEDPlus/JST7580 (JDreamIID, CAplus/MEDLINE/EMBASE/BIOSIS (STN), WPIIOS/WPIIX (STN))			
C. 関連すると認められる文献			
引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求項の番号	
A	WO 2016/024195 A1 (NOVARTIS AG) 2016.02.18, 請求項 1-61, 実施例特に実施例 7, 図 4 等 & US 2016/0046711 A1, 請求項 1-61, 実施例特に実施例 7, 図 4 等	1-53	
A	WO 2014/057687 A1 (第一三共株式会社) 2014.04.17, 請求項 1-49, 実施例等 & US 2015/0297748 A1, 請求項 1-49, 実施例等	1-53	
☐ C欄の続きにも文献が列挙されている。 ☐ パテントファミリーに関する別紙を参照。			
* 引用文献のカテゴリー 「A」 特に関連のある文献ではなく、一般的技術水準を示すもの 「E」 国際出願日前の出願または特許であるが、国際出願日以後に公表されたもの 「L」 優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献 (理由を付す) 「O」 口頭による開示、使用、展示等に言及する文献 「P」 国際出願日前で、かつ優先権の主張の基礎となる出願日の後に公表された文献 「T」 国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの 「X」 特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの 「Y」 特に関連のある文献であって、当該文献と他の 1 以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの 「&」 同一パテントファミリー文献			
国際調査を完了した日 30.07.2018		国際調査報告の発注日 07.08.2018	
国際調査機関の名称及びあて先 日本国特許庁 (ISA/J P) 郵便番号 100-8915 東京都千代田区霞が関三丁目 4 番 3 号		特許庁審査官 (権限のある職員) 田ノ上 拓自 電話番号 03-3581-1101 内線 3448	4B 6115

国際調査報告

国際出願番号 PCT/JP2018/018572

発明の属する分野の分類

C12N15/13(2006.01)i, A61K9/00(2006.01)i, A61K31/4745(2006.01)i,
A61K39/395(2006.01)i, A61K47/68(2017.01)i, A61P13/12(2006.01)i, A61P15/08(2006.01)i,
A61P35/00(2006.01)i, A61P43/00(2006.01)i, C07K16/28(2006.01)i, C12N1/15(2006.01)i,
C12N1/19(2006.01)i, C12N1/21(2006.01)i, C12N5/10(2006.01)i, C12P21/08(2006.01)i

特許協力条約

発行人 日本国特許庁（国際調査機関）

代理人 石橋 公樹 印 あて名 〒140-0065 日本国東京都品川区広町一丁目2番58号 第一三 共株式会社内		PCT 国際調査機関の見解書 (法施行規則第40条の2) [PCT規則43の2.1]	
		発送日 (日, 月, 年) 07. 08. 2018	
出願人又は代理人 の書類記号 BSPCT-PP1814		今後の手続については、下記2を参照すること。	
国際出願番号 PCT/JP2018/018572	国際出願日 (日, 月, 年) 14. 05. 2018	優先日 (日, 月, 年) 15. 05. 2017	
国際特許分類 (IPC) IntCl 補充欄参照			
出願人 (氏名又は名称) 第一三共株式会社			

1. この見解書は次の内容を含む。

- ☒ 第I欄 見解の基礎
- ☐ 第II欄 優先権
- ☐ 第III欄 新規性、進歩性又は産業上の利用可能性についての見解の不作成
- ☐ 第IV欄 発明の単一性の欠如
- ☒ 第V欄 PCT規則43の2.1(a)(i)に規定する新規性、進歩性又は産業上の利用可能性についての見解、それを裏付けるための文献及び説明
- ☐ 第VI欄 ある種の引用文献
- ☐ 第VII欄 国際出願の欠陥
- ☐ 第VIII欄 国際出願についての意見

2. 今後の手続

国際予備審査の請求がされた場合は、出願人がこの国際調査機関とは異なる国際予備審査機関を選択し、かつ、その国際予備審査機関がPCT規則66.1の2(b)の規定に基づいて国際調査機関の見解書を国際予備審査機関の見解書とみなさない旨を国際事務局に通知していた場合を除いて、この見解書は国際予備審査機関の最初の見解書とみなされる。

この見解書が上記のように国際予備審査機関の見解書とみなされる場合、様式PCT/ISA/220を送付した日から3月又は優先日から22月のうちいずれか遅く満了する期限が経過するまでに、出願人は国際予備審査機関に、適当な場合は補正書とともに、答弁書を提出することができる。

さらなる選択肢は、様式PCT/ISA/220を参照すること。

見解書を作成した日 30. 07. 2018			
名称及びあて先 日本国特許庁 (ISA/JP) 郵便番号100-8915 東京都千代田区霞が関三丁目4番3号		特許庁審査官 (権限のある職員) 田ノ上 拓自 電話番号 03-3581-1101 内線 2448	4B 6115

様式PCT/ISA/237 (表紙) (2015年1月)

国際調査機関の見解書	国際出願番号 PCT/J P 2018/018572
第1欄 見解の基礎	
<p>1. 言語に関し、この見解書は以下のものに基づき作成した。</p> <p>☒ 出願時の言語による国際出願</p> <p>☐ 出願時の言語から国際調査のための言語である _____ 語に翻訳された、この国際出願の翻訳文 (PCT規則12.3(a)及び23.1(b))</p> <p>2. ☐ この見解書は、PCT規則91の規定により国際調査機関が許可した又は国際調査機関に通知された明らかな誤りの訂正を考慮して作成した (PCT規則43の2.1(b))。</p> <p>3. この国際出願で開示されたスクリーンショット又はアミノ酸配列に関して、以下の配列表に基づき見解書を作成した。</p> <p>a. ☒ 出願時における国際出願の一部を構成する配列表</p> <p style="margin-left: 20px;">☒ 附属書C/ST.25テキストファイル形式</p> <p style="margin-left: 20px;">☐ 紙形式又はイメージファイル形式</p> <p>b. ☐ 国際出願とともに、PCT規則13の3.1(a)に基づき国際調査のためにのみ提出された、附属書C/ST.25テキストファイル形式の配列表</p> <p>c. ☐ 国際出願日後に、国際調査のためにのみ提出された配列表</p> <p style="margin-left: 20px;">☐ 附属書C/ST.25テキストファイル形式 (PCT規則13の3.1(a))</p> <p style="margin-left: 20px;">☐ 紙形式又はイメージファイル形式 (PCT規則13の3.1(b)及びPCT実施細則第713号)</p> <p>4. ☐ さらに、複数の版の配列表又は配列表の写しが提出され、変更後の配列表又は追加の写しに記載された情報が、出願時における配列表と同一である旨、又は出願時における国際出願の開示の範囲を超えない旨の陳述書の提出があった。</p> <p>5. 補足意見:</p>	

国際調査機関の見解書		国際出願番号 PCT/J P 2018/018572	
第V欄 新規性、進歩性又は産業上の利用可能性についてのPCT規則43の2.1(a) (i)に定める見解、それを裏付ける文意及び説明			
1. 見解			
新規性 (N)	請求項 請求項	1-53 _____	有 無
進歩性 (I S)	請求項 請求項	1-53 _____	有 無
産業上の利用可能性 (I A)	請求項 請求項	1-53 _____	有 無
2. 文献及び説明			
<p>文献1 : WO 2016/024195 A1 (NOVARTIS AG) 2016.02.18, 請求項 1-61, 実施例特に実施例7, 図4等 & US 2016/0046711 A1, 請求項 1-61, 実施例特に実施例7, 図4等</p> <p>文献2 : WO 2014/057687 A1 (第一三共株式会社) 2014.04.17, 請求項 1-49, 実施例等 & US 2015/0297748 A1, 請求項 1-49, 実施例等</p> <p>請求項 1-53に係る発明は、国際調査報告にて引用された文献に対して新規性及び進歩性を有する。</p> <p>文献1には、NOVO0712等のカドヘリン6に対する抗体が記載されており(請求項 1-61、実施例等参照)、NOVO0712はカドヘリン6のEC5に結合することが記載されている(実施例7、図4等参照)。また、該抗体を卵巣癌等の治療に使用することも記載されている(請求項 40等参照)。</p> <p>文献2は、いわゆるGGFGリンカーに関するものであり、本願の請求項 31-36に記載されるリンカー化合物が記載されている(請求項 1-49、実施例等参照)。</p> <p>しかしながら、文献1-2のいずれにも、本願の配列番号4、すなわちカドヘリン6のEC3に特異的に結合する抗体は記載も示唆もされておらず、また、本願実施例にて示される、良好な内在化能についても記載も示唆もされていない。さらに、本願実施例では、カドヘリン6のEC3に特異的に結合する抗体が、先行技術たる文献1のNOVO0712と比較して、より高い内在活性及び細胞傷害活性を有することも示されている。</p> <p>したがって、本願請求項 1-53に係る発明は、国際調査報告にて引用されたいずれの文献にも記載も示唆もされていないものであり、出願時の技術常識を考慮しても、当業者といえども容易には想到し得ないものである。</p>			

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Isolation and Sequence Analysis of Human Cadherin-6 Complementary DNA for the Full Coding Sequence and Its Expression in Human Carcinoma Cells¹

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ABSTRACT

The expression pattern of E- and P-cadherin in human carcinomas has been reported by many laboratories. However, little is known about the involvement of other cadherin types in human carcinomas. cDNA clones for a cadherin molecule were isolated from a cDNA library of human hepatocellular carcinoma cells which lacked E- and P-cadherin expression but exhibited cell aggregation activity mediated by an unknown cadherin, and they were subjected to sequence analysis. The overlapped clones covered 4315 nucleotides and were found to encode a typical cadherin molecule consisting of 790 amino acids. Since the deduced amino acid sequence was identical to a partially available human cadherin-6 sequence except for two amino acid residues, the clones were considered to be human cadherin-6 cDNAs encoding the entire open reading frame. The deduced amino acid sequence also showed extremely high homology with recently reported rat K-cadherin, 97% for the putative mature protein, suggesting that cadherin-6 is the human counterpart of rat K-cadherin. Expression of cadherin-6 in various human normal tissues and carcinoma cells was examined by Northern blot analysis using a specific probe corresponding to the signal and precursor sequence. Among normal tissues examined, brain, cerebellum, and kidney showed strong expression of cadherin-6, whereas lung, pancreas, and gastric mucosa showed weak expression. Transcripts of cadherin-6 were not detected in normal liver, whereas four of six hepatocellular carcinoma cell lines examined expressed cadherin-6 abundantly. As reported for rat K-cadherin, three renal carcinoma cell lines also expressed cadherin-6 strongly. The most interesting finding was obtained for small cell lung carcinoma lines. Among 15 of such cell lines examined, all of 11 cadherin-6-positive lines were classified into the classic type, whereas the negative cell lines were all of the variant type. The present results suggest that besides E- and P-cadherin, other cadherin molecules are expressed in human cancers and are responsible for additional biological properties of the carcinoma cells.

INTRODUCTION

Cadherin is a Ca^{2+} -dependent cell-cell adhesion molecule which mediates cell-cell binding in a homophilic manner. Cadherin constitutes a gene family and consists of subclasses with individual binding specificities. Initial studies identified three subclasses, epithelial (E-), neural (N-) and placental (P-) cadherin, and further characterization revealed that they played a key role in many morphogenetic events as well as in the maintenance of orderly structures such as epithelium (reviewed in Refs. 1 and 2). We started studying human cadherins, focusing on their possible involvement in the biological properties of cancer, since it was conceivable that metastasis and invasion of cancer could not occur without disruption of the mutual adhesion of cancer cells (3-6).

To date, a considerable volume of experimental and clinical evidence has been obtained to indicate that dysfunction of E-cadherin, a major cadherin molecule expressed in both epithelium and cancer,

leads at least to enhanced invasiveness and progression of cancer (4, 5, 7-12), and it has become apparent that there are a number of mechanisms responsible for the E-cadherin dysfunction in cancer, including loss or reduction of expression, mutations (13, 14), and aberrations of catenins (6, 15-22), which connect cadherin to the cytoskeleton network.

Besides the three subclasses mentioned above, many molecules with characteristics of cadherin have been identified over the past few years. These include the typical cadherins, which possess the same domain structure (23-26), a truncated-type cadherin (27), cadherin-related proteins with more than five extracellular subdomains (28), and desmosomal cadherins (29-31), indicating the existence of a cadherin superfamily. In addition, a PCR technique based on the high conservation among cadherin family members has facilitated the molecular cloning of various cadherins (32-34). Because E- and P-cadherin are thus far the only two cadherins that have been well characterized in cancer, it would be of interest to determine whether other cadherins are expressed in cancer and affect its biological properties. Before undertaking the present study, we had noticed some cancer cell lines exhibiting strong cell aggregation activity after trypsin/ Ca^{2+} treatment (35) without expression of E- and P-cadherin (data not shown), suggesting that other cadherin molecule(s) were present in them. In this report, we describe the molecular cloning and sequence analysis of a cadherin molecule from one of those lines, a human hepatocellular carcinoma cell line Li21 (36), and its unique expression pattern in cancer cells.

MATERIALS AND METHODS

PCR. To obtain a cDNA fragment of the unknown cadherin expressed in Li21 cells, PCR was performed based on a strategy similar to that described by Suzuki *et al.* (32). The primer pair used was the upstream primer 5'-GGA-ATTAC(ACGT)GC(ACGT)CC(ACGT)CC(ACGT)TA(CT)GA-3' and the downstream primer GGAATTCTC(ACGT)GC(ACGT)A(AG)(CT)TT(CT)-TT(AG)AA-3', both of which contained an *EcoRI* recognition site at their 5' ends. Poly(A)⁺ RNA was prepared from C-Li21 cells as described by Okayama *et al.* (37). Single-strand cDNA was synthesized using Superscript II (GIBCO-BRL, Gaithersburg, MD) and subjected as a template to 35 cycles of PCR (94°C, 55°C, and 72°C for 1.5, 2, and 3 min, respectively). The PCR product, about 160 bp long, was purified, digested with *EcoRI*, and subcloned into the *EcoRI* site of the pBluescript II SK(-) phagemid vector. This fragment was used as a probe for cDNA cloning as described below.

Construction and Screening of cDNA Library. An oligo-dT-primed cDNA was synthesized from poly(A)⁺ RNA of Li21 cells using a cDNA synthesis system plus kit (Amersham, Buckinghamshire, United Kingdom), ligated to the arms of λ gt10 via *EcoRI* adaptors, and packaged *in vitro* using the cDNA cloning system, λ gt10 (Amersham). The phage plaques were transferred to nylon filters. The filters were hybridized at 42°C in a buffer containing 50% formamide, 0.65 M NaCl, and the above PCR fragment labeled with [α -³²P]dCTP, washed twice with 0.1X SSC-0.1% SDS at 65°C for 30 min, and exposed to Kodak XAR-5 film (Rochester, NY) at -70°C with an intensifying screen. Positive clones were plaque purified under the same conditions as those above.

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³ The abbreviations used are: poly(A)⁺, polyadenylated; bp, base pair(s); SCLC, small cell lung cancer; N-CAM, neural cell adhesion molecule.

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DNA Sequence Analysis. The inserts of the positive clones were subcloned into pBluescript II SK(-) or pUC118 (Takara Shuzo, Kyoto, Japan). The phagemids were denatured by alkali treatment and subjected to sequence analysis using a 7-deaza-GTP Sequenase version 2 kit (United States Biochemicals, Cleveland, OH). The DNA sequence was determined on both strands successively using oligonucleotide primers which anneal the vector and insert DNA sequences. The nucleotide and amino acid sequences were analyzed using the GeneWorks software package (IntelliGenetics, CA).

Northern Blot Analysis. Total RNA was prepared from various human tissues and cancer cell lines by acid guanidinium thiocyanate-phenol-chloroform extraction (38), and poly(A)⁺ RNA was isolated using oligo-dT latex beads (Oligotex-dT30 Super; Daiichikagaku, Tokyo, Japan). Human brain, cerebellum, and pancreas poly(A)⁺ RNAs were purchased from Clontech (Palo Alto, CA). Poly(A)⁺ RNAs (2 µg/lane) were separated in 1% agarose/formaldehyde gels, transferred to nitrocellulose filters, and hybridized at 42°C in a buffer containing 50% formamide and 0.65 M NaCl with a specific PCR probe described below. To avoid cross-hybridization with other cadherin subclasses, a 182-bp nucleotide sequence (positions 116–297 in Fig. 1) encoding the signal peptide and precursor region, where significant homologies have not been reported among different subclasses, was amplified by a PCR-labeling procedure (13) and used as a probe. We confirmed that this probe did not detect either E- or P-cadherin (data not shown). The filters were washed twice with 2X SSC, 20 mM sodium phosphate buffer, 0.06% sodium PP₆, and 0.05% SDS at 65°C for 30 min and exposed to XAR-5 film at -70°C with an intensifying screen.

RESULTS

Amplification and Isolation of a Cadherin cDNA Fragment. A PCR method was used to isolate a cDNA fragment of an unknown cadherin expressed in Li21 cells using a set of two degenerate oligonucleotide primers encoding highly conserved sequences in the cytoplasmic domain of known cadherins (32), and a fragment about 160 bp long was obtained (data not shown). This fragment was subcloned into pBluescript II SK(-) and subjected to sequence analysis. The DNA sequence consisted of 155 nucleotides and showed significant homology with reported cDNA sequences of known cadherins (data not shown).

cDNA Cloning and Sequencing. The λgt10 cDNA library constructed from Li21 poly(A)⁺ RNA was screened with the 155-bp fragment. Approximately 4×10^4 recombinants were screened, and only one positive clone, designated AL1-1, was isolated. This clone contained a cDNA insert of 3787 bp with the poly(A) sequence, but comparison with cDNA sequences of known cadherins revealed that it lacked the translation initiation codon and the following nucleotides encoding the signal peptide, precursor region, and most of extracellular domain 1. In order to identify this sequence, the library was screened again using a 321-bp nucleotide located at the 5'-end of AL1-1 (positions 650–970 in Fig. 1) amplified by PCR as a probe. Approximately 2×10^5 recombinants were rescreened, and three positive clones were isolated. Among these clones, one, designated

B1-1, proved to contain the entire open reading frame. Although B1-1 also had a poly(A) tail at the 3'-end, the addition site was different from that of AL1-1. Fig. 1 shows the two clones schematically with representative restriction endonuclease sites.

The overlapping nucleotide sequence, 4315 bp in length, is shown in Fig. 2. The open reading frame begins with an ATG codon at position 121–123, terminates at a TAA codon at position 2491–2493, and consists of 2370 nucleotides encoding 790 amino acids. A polyadenylation signal, ATTAAA, is identified at position 3101–3106 for clone B1-1, but no obvious signal is present for AL1-1. The deduced amino acid sequence contains two hydrophobic sequences corresponding to the signal peptide and transmembrane domain, the long extracellular domain, and the short cytoplasmic domain, which are structures common to typical cadherins (1). This polypeptide is expected to undergo posttranslational modifications including proteolytic cleavage at amino acid position 53–54 (39) and to be expressed at the cell surface as the mature protein, which is 737 amino acids long with 5 consensus sites for N-linked glycosylation (40).

Comparison with Other Cadherins. A homology search with cadherin sequences reported previously revealed that this cadherin shows striking identity with two cadherin sequences. One of these is cadherin-6, which has been cloned from a human fetal brain library and partially sequenced (32). The amino acid sequence of the present cadherin from position 377 to 790 completely agrees with the reported 424 amino acids of cadherin-6, except for only two amino acid residues (Val at position 421 and Thr at position 425 are both Ile in cadherin-6), strongly suggesting that the clones presented here encode cadherin-6. To avoid confusion, the cadherin cloned in the present study will be referred to as cadherin-6 hereafter. The other cadherin highly homologous with this cadherin-6 is rat K-cadherin, which was recently isolated from a rat renal cell carcinoma library (41). Homologies of cadherin-6 with rat K-cadherin and other known human cadherins are summarized in Table 1. Homology between cadherin-6 and rat K-cadherin is extremely high, being 97% for the putative mature protein, as compared with those between cadherin-6 and known human cadherins. Even for the signal and precursor sequences, where each cadherin subclass shows a characteristic sequence, the two cadherins are markedly homologous, suggesting that cadherin-6 is the human counterpart of rat K-cadherin.

Comparison of cadherin-6 with other human cadherin subclasses reveals that there are two groups of cadherin on the basis of resemblance to cadherin-6. One group includes cadherin-8, -11 and -12, which exhibit over 60% homology with cadherin-6, and when aligned with cadherin-6, few gaps are required. The other group includes E-, N- and P-cadherin, and cadherin-4 and -5, which show lower homology, 30–40%, with cadherin-6. Cadherin-13, which lacks the transmembrane and cytoplasmic regions, also shows lower homology, 30%, for the extracellular part.

Expression of Cadherin-6 in Human Tissues and Carcinoma Cell Lines.

To examine cadherin-6 expression by RNA blotting without cross-hybridization with other cadherin species, we chose a 182-bp fragment corresponding to the signal and precursor sequences as a probe. Fig. 3 shows cadherin-6 expression in Li21 cells and various normal human tissues. Cadherin-6 transcripts were detected as four bands intensely in Li21 cells, brain, cerebellum, and kidney and faintly in lung, pancreas, and gastric mucosa, with approximate molecular sizes of 9, 4.1, 3.4, and 2.7 kb. This result is inconsistent with the finding by Xiang *et al.* (41) that K-cadherin was scarcely expressed in adult rat tissues and also the report by Suzuki *et al.* (32) that brain expressed cadherin-6 very faintly. Interestingly, expression of the 9-kb cadherin-6 transcript was very faint in Li21 cells and kidney but intense in brain and cerebellum, and expression of the 2.7-kb transcript was also faint in cerebellum and kidney, indicating that each

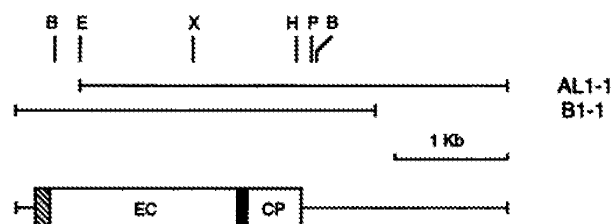


Fig. 1. Two cDNA clones, AL1-1 and B1-1, and their restriction map with a schematic drawing of cadherin structure. The box indicates the coding region. Hatched, solid, and clear areas represent the signal and precursor sequence, the transmembrane domain, and the extracellular (EC) and cytoplasmic (CP) domains, respectively. B, BamHI; E, EcoRI; H, HindIII; P, PstI; X, XbaI.

tissue or cell type might show preferential selection of the polyadenylation site. Cadherin-6 mRNA was not detected in poly(A)⁺ RNA isolated from heart, liver, and colonic mucosa.

Cadherin-6 expression in various human carcinoma cell lines was then examined using an approach similar to that described above. RNA blotting of five hepatocellular carcinoma cell lines, except for Li21, is shown in Fig. 4A. Li22, Li23, and Li24 cells (36) strongly expressed 4.1-, 3.4-, and 2.7-kb cadherin-6 transcripts. In contrast, no bands were detected in Li7 (42) and HepG2 (43). Consequently, four of six hepatoma lines strongly expressed cadherin-6. Among four renal cell carcinoma cell lines examined, three expressed cadherin-6 (Fig. 4B), which is consistent with the previous finding that K-cadherin was expressed in one of three human renal cell carcinomas (41). Most of the SCLC cell lines examined (11 of 15) also expressed cadherin-6 with various degrees of intensity, although cadherin-6 mRNA was not detected in four lines, Lu24v, Lu135v, H82, and N417 (Refs. 44 and 45; Fig. 4C). Besides the human carcinoma cells described here, we examined cadherin-6 expression in various human cell lines including five colon adenocarcinomas, four gastric adenocarcinomas, two pancreatic adenocarcinomas, and one cell line each of lung adenocarcinoma, esophageal squamous carcinoma, vulvar squamous carcinoma, cervical carcinoma, bladder carcinoma, retinoblastoma, and transformed endothelium. Cadherin-6 transcripts were not, or only very weakly detected, in poly(A)⁺ RNAs from these lines (data not shown).

DISCUSSION

We have reported here cloning of a human cadherin molecule from a cDNA library of a hepatocellular carcinoma cell line, Li21, which does not express either E- or P-cadherin but exhibits cell aggregation mediated by an unknown cadherin. The deduced amino acid sequence is essentially identical to that of partially identified human cadherin-6 (32), except that Val at amino acid position 421 and Thr at 425 are both replaced by Ile in the latter. Since it is unlikely that two different genes separately encode the two molecules, we conclude that the cDNA clones presented here encode human cadherin-6 and that these are the first reported clones which cover the entire open reading frame. It has been reported that human N-cadherin cDNAs isolated from different libraries show minor differences from one another (46–48). The two amino acid substitutions found here may be explained by polymorphism, mutation, or cloning artifacts. It is noteworthy that, at these two positions, a cDNA clone of rat K-cadherin, isolated from a renal cell carcinoma library (41), encodes the same amino acids as AL1-1 and B1-1, suggesting that the two amino acid substitutions may be a characteristic of cancer-specific cadherin-6.

Suzuki *et al.* (32) and Tanihara *et al.* (34) have proposed that typical cadherins can be divided into two types on the basis of structural similarities: type I including E-, N-, P-, B- (23), R- (24), EP- (25), and M-cadherin (26), and cadherin-4; and type II cadherins including cadherin-5, -8, -11, and -12. They also suggested that cadherin-6, -7, -9, and -10, for which only restricted sequences are available, might be classified as type II cadherins (32, 34). The entire cadherin-6 sequence presented here shows higher homology with cadherin-8, -11, and -12 than with other human cadherins (Table 1), confirming that cadherin-6 should be a type II cadherin. However, only cadherin-5 among type II cadherins shows a low similarity to

cadherin-6, comparable to the similarity between cadherin-6 and type I cadherins (Table 1). This is consistent with the previous finding that cadherin-5 shows rather low similarity to other type II cadherins (32), suggesting that cadherin-5 is a unique molecule among the type II cadherins.

Since human cadherin-6 shows extremely high similarity to rat K-cadherin (41), even in the signal peptide and precursor regions, it is considered to be the human counterpart of rat K-cadherin. Interestingly, the most diversified region between the two cadherins is the cytoplasmic domain, where two clusters of amino acids different from each other are present, whereas the two cadherins are almost identical in the extracellular and transmembrane domains with only a few conservative amino acid changes (Fig. 2). In contrast, the classical-type cadherins, E-, N- and P-cadherin, show the highest degree of interspecific conservation in the cytoplasmic domain (48–51), which is linked to the cytoskeleton via catenins. When each subdomain of cadherin-6 is compared with that of other type II molecules, the most conserved subdomain is not the cytoplasmic domain but the extracellular domain 2 (Table 1). As for the extracellular domain of cadherin-6, three potential sequences involved in cell adhesion recognition, PPI, GAD and HAV, proposed by Blaschuk *et al.* (52), are absent or partially changed in cadherin-6. In contrast, possible Ca²⁺-binding sites (53, 54) are well conserved in cadherin-6, and four cysteine residues involved in the mechanism of cadherin adhesion (55) are also conserved in cadherin-6 at amino acid positions 497, 589, 591, and 600 (Fig. 1). These features are also the case for the rat homologue, K-cadherin (41). As mentioned above, cadherin-6 and other type II cadherins have structural characteristics not only similar to but also different from classic cadherins. It is thus of considerable interest to investigate whether cadherin-6 and other type II cadherins function in the same manner as classic cadherins.

Analysis of the expression pattern of cadherin-6 revealed in this study provided some interesting findings. Both normal kidney and renal cell carcinomas expressed cadherin-6. In contrast, although four of six hepatocellular carcinoma cell lines examined strongly expressed cadherin-6, and indeed cadherin-6 cDNAs were isolated from a hepatocellular carcinoma library, cadherin-6 expression was not detected in normal liver tissue. The significance of the cadherin-6 expression in hepatocellular carcinoma cells is still unclear; therefore, it will be important in the future to examine cadherin-6 expression in surgically resected hepatomas together with the morphological and clinical features of the cancers.

Since brain and cerebellum strongly expressed cadherin-6, we focused on cadherin-6 expression in SCLC cells, which are known to show features of differentiation similar to neuroendocrine cells. SCLC cells can be subdivided into two types, the classic type and the variant type, according to the expression pattern of neuroendocrine biomarkers, and separately subgrouped into four types based on their appearance: type 1, tightly packed spherical aggregates; type 2, relatively densely packed aggregates; type 3, very loosely adherent aggregates; and type 4, cells attached to the substrate (45). As shown in Fig. 4C, cadherin-6 expression was detected in 11 of 15 SCLC lines examined. Surprisingly, all of these 11 cadherin-6-positive cell lines were of the classic type and morphologically type 1 or 2. In contrast, all four of the cadherin-6-negative cell lines were classified as the variant type and were morphologically type 3 (only Lu24v cells grow as type 2–3;

Fig. 2. Nucleotide sequence (upper) and deduced amino acid sequence (lower) of a cadherin molecule cloned from a Li21 cDNA library. Amino acid sequence is shown in one-letter code. The stop codon is marked by an asterisk. The addition site of the poly(A) tail and the polyadenylation signal of clone B1-1 are shown by single and double underlining, respectively. The putative signal peptide and transmembrane region are also indicated by underlining. The solid triangle and clear triangles show the possible proteolytic cleavage site (39) and N-linked glycosylation sites, respectively. Capital and small letters below the deduced amino acid sequence indicate amino acid residues of rat K-cadherin (41) and human cadherin-6 partially sequenced by Suzuki *et al.* (32), respectively, which differ from the sequence presented here. The nucleotide sequence data will appear in the GSDB/DBJ/EMBL/NCBI databases under the accession number D31784.

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Refs. 44 and 45). These findings suggest that the expression of cadherin-6 is associated with neuroendocrine differentiation and that cadherin-6 may be a useful marker for distinguishing between the classic and variant types; furthermore, cadherin-6 expression may be responsible for the tighter cell-cell adhesion evident in SCLC cell lines positive for the molecule than in those lacking it. A similar finding has been reported for the Ca^{2+} -independent cell-cell adhesion molecule N-CAM, also called cluster 1 SCLC antigen; N-CAM was abundantly expressed in classic-type SCLCs, whereas the expression

Table 1 Homologies between cadherin-6 and other cadherins for separate regions and the putative mature protein

The extracellular domain is divided into five subregions according to Tanihara *et al.* (34). rK, E, N, P, 4, 5, 8, 11, 12, and 13 represent percentage homology between human cadherin-6 and rat K-cadherin (41), human E-cadherin (49), N-cadherin (46), P-cadherin (50), and cadherin-4, -5, -8, -11, -12, and -13 (32, 34), respectively. EC, extracellular domain; TM, transmembrane domain; CP, cytoplasmic domain.

	EC1	2	3	4	5	TM	CP	Total
rK	100	98	97	98	100	100	89	97
E	29	43	31	29	23	29	50	35
N	34	47	27	31	24	50	48	37
P	29	37	33	21	17	26	49	32
4	31	46	30	29	24	49	46	36
5	38	49	37	41	37	41	35	39
8	60	75	57	57	51	64	60	60
11	62	78	52	53	48	73	62	60
12	70	78	62	43	56	73	71	64
13	32	38	30	28	21			30 ^a

^a Because cadherin-13 lacks the transmembrane and cytoplasmic domains, only the homology in the extracellular domain is shown.

1 2 3 4 5 6 7 8 9 10 11

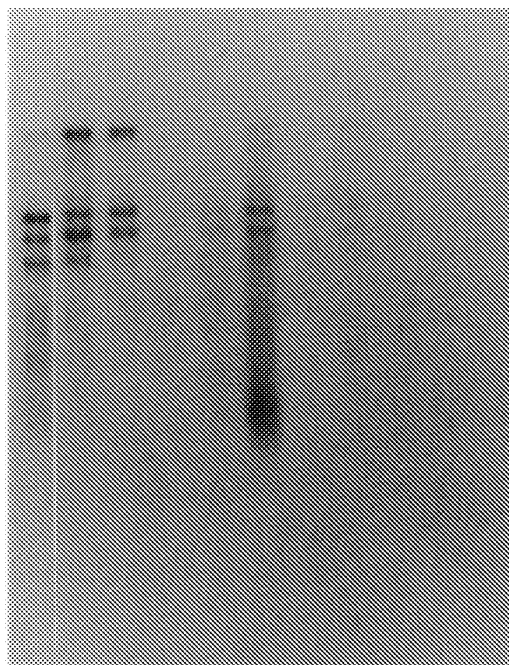


Fig. 3. Expression of cadherin-6 in Li21 cells and normal human tissues examined by Northern blot analysis. Poly(A)⁺ RNA from Li21 cells (Lane 1), human brain (Lane 2), cerebellum (Lane 3), heart (Lane 4), lung (Lane 5), kidney (Lane 6), liver (Lane 7), pancreas (Lane 8), gastric mucosa (Lane 9), colonic mucosa (Lane 10), and placenta (Lane 11) were hybridized with the 182-bp PCR probe. The RNA sample in Lane 6 appears to be degraded in comparison with other samples, probably because of the prolonged ischemic state of the kidney tissue during surgical resection. Bars show positions of molecular size marker mRNAs of 9.5, 7.5, 4.4, 2.4, 1.4, and 0.24 kb from the top.

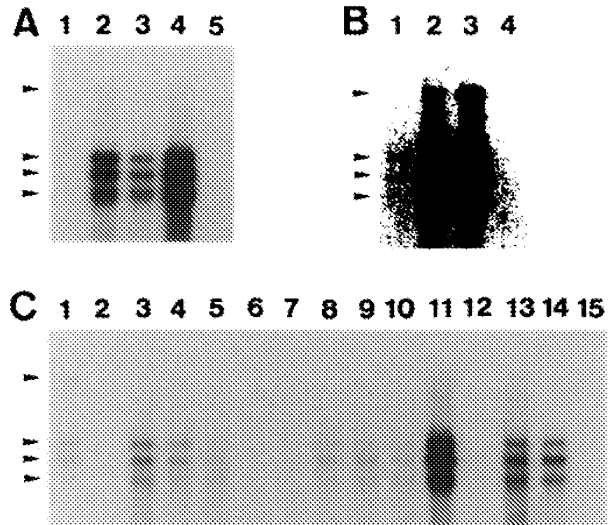


Fig. 4. Expression of cadherin-6 in hepatocellular carcinoma cell lines (A), renal cell carcinoma cell lines (B) and SCLC cell lines (C) examined by Northern blot analysis. A: Lane 1, Li7; Lane 2, Li22; Lane 3, Li23; Lane 4, Li24 (36, 42); Lane 5, Hep G2 (43). B: Lane 1, KT4; Lane 2, KT12 (57); Lane 3, KT12 (57); Lane 4, KT35. C: Lane 1, Lu24; Lane 2, Lu24v; Lane 3, Lu130; Lane 4, Lu134A; Lane 5, Lu134B; Lane 6, Lu135v; Lane 7, Lu139; Lane 8, Lu140; Lane 9, Lu141; Lane 10, Lu143 (44); Lane 11, H69; Lane 12, H82; Lane 13, N230; Lane 14, N231; Lane 15, N417 (45). Arrowheads, positions of cadherin-6 transcripts.

level tended to be lower in the variant type (56). It has also been reported that in kidney development, the expression pattern of K-cadherin, a homologue of cadherin-6, is similar to that of N-CAM (41). However, it remains to be elucidated whether the expressions of both cadherin-6 and N-CAM are regulated by the same mechanisms and whether their expression actually affects the morphological appearance and biological properties of SCLC cells.

In conclusion, we have isolated a full-length human cadherin-6 cDNA from a hepatocellular carcinoma library and demonstrated its unique expression in cancer cells. However, the exact functions and roles of cadherin-6 during normal morphogenesis and cancer development remains to be elucidated. We hope that this report will promote further studies on the roles of various cadherin molecules in cancer development and spread.

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Isolation and Sequence Analysis of Human Cadherin-6 Complementary DNA for the Full Coding Sequence and Its Expression in Human Carcinoma Cells

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Cadherin-6 Expression Transiently Delineates Specific Rhombomeres, Other Neural Tube Subdivisions, and Neural Crest Subpopulations in Mouse Embryos

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Mammalian cadherin-6 (K-cadherin, cad6) was originally identified by means of the polymerase chain reaction, but its biological functions have not yet been determined. We analyzed the expression pattern of the mouse homologue of this cadherin during development and found that it was transiently expressed in restricted rhombomeres and in other subdivisions of the neural plate and tube. In the midbrain and anterior hindbrain of E8.0–8.5 embryos, cad6 was expressed only in neural crest-generating regions. In contrast, in the posterior hindbrain and contiguous spinal cord of these embryos, cad6 occurred throughout the neural plate, forming a sharp anterior limit at the future rhombomere 4 and 5 boundary. Subsequently, this neural plate expression became confined to rhombomere 6, although most of the neural crest-generating areas remained positive throughout the body. Neural crest cells expressing cad6 migrated out of the neural tube, and subsequently accumulated mainly along peripheral nerves. We then studied the effect of *Hoxa-1* mutation on the expression of cad6, as their expressions spatiotemporally overlapped with each other in the early posterior hindbrain. In E8.0–8.5 *Hoxa-1* mutants, cad6 expression was suppressed in the region of rhombomeres 4 to 6, although that in the other regions was not essentially affected. At later stages, however, cad6-positive crest cells appeared and migrated out of rhombomeres 4 to 6, indicating that the suppression of cad6 expression was transient and restricted to early stages. Importantly, this effect of the *Hoxa-1* mutation concurred with the timing of the expression of this gene. We also studied *Hoxa-3* mutants, but found no effect of this mutation on the cad6 expression pattern. These findings suggest that cad6 may contribute to the formation of the segmental structure of the early brain through its ability to confer specific adhesiveness on cells and that *Hoxa-1* may be required for early cad6 expression in the posterior hindbrain. © 1997 Academic Press

INTRODUCTION

Cadherins represent a superfamily of transmembrane or surface-anchored proteins characterized by the presence of unique repeated motifs on their extracellular domain (Ranscht, 1994). Members of one subfamily, called classic cadherins, have been identified as homophilic cell–cell adhesion molecules (Takeichi, 1988). They are subdivided into two groups, types I and II (Suzuki *et al.*, 1991; Takeichi, 1995). The type-I group includes E-, N-, and R-cadherin, and the type-II group includes cadherin-5 to -12. Each of these molecules is expressed in a unique pattern in the body, and dysfunction of them causes defective morphogenesis of tissues, as demonstrated by a number of studies. For example, blocking of E-cadherin disorganizes preimplantation embryos (Larue *et al.*, 1994; Riethmacher *et al.*, 1995) as

well as differentiated epithelial layers (Hirai *et al.*, 1989; Hermiston *et al.*, 1995), and that of N-cadherin impairs neural morphogenesis (Matsunaga *et al.*, 1988; Barami *et al.*, 1994). With respect to homophilic interactions between the classic cadherins, like subtypes prefer to bind together (Takeichi, 1995), although heterotypic interactions were observed between certain cadherin subtypes (Murphy-Erdosh *et al.*, 1995). As a result, cells expressing a particular cadherin selectively adhere to those expressing the same cadherin. It was thus proposed that cadherins are essential not only for physical linking of cells but also for selective association of different cell types.

Two kinds of morphogenetic events, likely relevant to the above functions of cadherins in selective cell association, were recently reported. First, we found that two type-II cadherins, cadherin-6B and cadherin-7, were expressed

during specific steps of neural crest development in the chicken embryo (Nakagawa and Takeichi, 1995). The chicken cadherin-6B (cad6B) is exclusively expressed in the future neural crest regions of the neural fold. When neural crest cells begin migration, they cease the expression of cad6B and instead begin to express cadherin-7 (cad7). This cad7 expression occurs in a subpopulation-specific manner. For example, in the trunk, cad7 is expressed only in crest cells populating the dorsal and ventral roots and spinal nerves, whereas it is never intensely expressed in the cranial and dorsal root ganglia or in the cranial mesenchyme, where neural crest cells are known to populate. Based on these and other observations, we hypothesized that neural crest cells migrate while maintaining their cadherin-mediated associations and that cad7 serves for the sorting of particular subsets of neural crest cells from the other subpopulations. As for the second morphogenetic event indicating cadherin-dependent selective cell association, R-cadherin was found to be expressed in a restricted fashion in specific rhombomeres of the embryonic chicken or mouse brain (Ganzler and Redies, 1995; Redies, 1995; Matsunami and Takeichi, 1995), some of which corresponded to certain "neuromeres." Cell aggregation assays conducted *in vitro* showed that cells derived from R-cadherin-positive and -negative regions segregated from each other in a cadherin-dependent manner. These findings led us to propose that R-cadherin confers specific aggregating ability on cells belonging to restricted subdivisions of the fetal brain, so as to confine them to the subdivisions. We argued that this mechanism may contribute to the formation or maintenance of the segmented structure of the fetal brain (Matsunami and Takeichi, 1995). It was also reported that a novel cadherin, F-cadherin, is expressed along several boundary regions in the *Xenopus* embryonic brain (Espeseth et al., 1995).

The above observations on the developing brains suggested that other cadherins may also be expressed in a segment-specific manner in the embryonic brain; this suggestion prompted us to search for such cadherins. It is known that neuroepithelial cells do not cross the boundaries between rhombomeres subdividing the hindbrain (Fraser et al., 1990), although a small percentage of them can (Birgbauer and Fraser, 1994), and that odd and even rhombomeres do not randomly intermix with each other (Clarke and Lumsden, 1993; Guthrie et al., 1993; Graham et al., 1993). These phenomena can be explained by assuming segment-specific adhesiveness of the cells comprising the hindbrain (Lumsden, 1990), and cadherins could be involved in this hypothetical mechanism. In the present study, we analyzed the expression pattern of mouse cadherin-6 (cad6) during embryogenesis, which is the mouse homolog of the previously cloned rat K-cadherin (Xiang et al., 1994) or human cadherin-6 (Shirayama et al., 1995). While various interesting expression patterns of cad6 were observed, one important finding was that it is transiently and sequentially expressed to delineate the boundaries between prospective rhombomeres 4 and 5, and then 5 and 6, providing the first molecular support of the notion that cadherin may endow hindbrain cells with rhombomere-specific adhesiveness. Be-

cause the cad6 expression pattern in the neural plate was reminiscent of that of *Hox* genes, we tested the possibility that the former may be regulated by these genes and found that in *Hoxa-1* mutants, the cad6 expression in the posterior hindbrain was temporarily suppressed.

MATERIALS AND METHODS

Animals

ICR mice were mated, and embryos at various stages were collected. The precise staging of collected embryos was made according to Theiler (1972). For *Hoxa-1* or *Hoxa-3* mutants, mice heterozygous for targeted disruptions of the *Hoxa-1* or *Hoxa-3* gene (Chisaka et al., 1991, 1992) were intercrossed to produce embryos, and their genotypes were determined by polymerase chain reaction (PCR) methods as described previously (Carpenter et al., 1993; Manley et al., 1995). The *Spotch* heterozygous mice were provided by Dr. S. Aizawa (Kumamoto University), and mated to produce embryos. Their genotypes were determined by their characteristic phenotype of the neural tube (Douglas et al., 1991).

Cloning of Mouse cad6 cDNA

To obtain cadherin cDNAs, we carried out PCR using mixed primers which can amplify multiple cadherin subtypes (Suzuki et al., 1991). As templates, cDNAs were prepared from mRNAs isolated from newborn mouse brains. By this PCR, about 160-bp fragments were amplified, and these fragments were subcloned into the pUC18 *EcoRI* site. To obtain full-length cDNAs, we constructed random primed λ gt10 cDNA libraries of postnatal brains derived from ICR mice by use of the cDNA Synthesis kit (Amersham). We screened these libraries with the above PCR fragments radiolabeled. Many overlapping clones were obtained, and among them one open reading frame (ORF) was found.

Probes for *In Situ* Hybridization

Sense or antisense cad6 RNA probes labeled with digoxigenin (Dig)-11-UTP (Boehringer-Mannheim) were prepared from pBSH1.0B, which contains the 202–1229 *Bam*HI fragment of cad6 cDNA. For the antisense probe, the *Eco*RI linearized vector was transcribed with T3 RNA polymerase (Gibco BRL); for the sense probe, the *Xba*I linearized vector was transcribed with T7 RNA polymerase (Gibco BRL). *Hoxa-1* antisense RNA probes labeled with Dig-11-UTP were prepared from a newly constructed pBlue-script vector containing the 1233–1950 *Eco*RI–*Bam*HI fragment of *Hoxa-1* cDNA (LaRosa and Gudas, 1988). For its antisense probe, the *Eco*RI-linearized vector was transcribed with T7 RNA polymerase. R-cadherin antisense RNA probes labeled with Dig-11-UTP and *Krox-20* antisense RNA probes labeled with fluorescein (FITC)-12-UTP (Boehringer Mannheim) were prepared, as described previously (Matsunami and Takeichi, 1995; Carpenter et al., 1993). All probes prepared were purified by use of Quick Spin Columns Sephadex G-50 (Boehringer-Mannheim), and stored in 50% formamide solutions at -20°C .

Whole-Mount *In Situ* Hybridization

Whole-mount *in situ* hybridization with a single probe was performed in the manner described previously (Shimamura et al.,

1994). For sliced samples, we fixed embryos overnight in PBS containing 4% paraformaldehyde (PFA) at 4°C and bleached them for 6 hr at room temperature in methanol containing one-fifth volume of 20% H₂O₂. After the samples had been rehydrated in a graded series of methanol/PBS, they were embedded in 2% agarose/PBS, and sectioned into 300- μ m slices by a microslicer (D.S.K. DTK-1000, Kyoto). For two-color, whole-mount *in situ* hybridization, both Dig-labeled and FITC-labeled RNA probes were added together after the pretreatment of samples, and incubation was carried out overnight. After nonbound probes had been removed by washing, the samples were incubated overnight with either anti-Dig-alkaline phosphatase (AP) or anti-FITC-AP conjugate; in general, we first used the less-sensitive probe, depending on the combination of the probes. In the case that the Dig probe was used first, we detected it with NBT-BCIP as a substrate to give a purple color. Then, we stopped the reaction with three changes of phosphate-buffered saline containing 0.1% Tween 20 (PB/T) and 1 mM EDTA and removed the first antibody conjugate by a few minutes' treatment in methanol. Next, we rehydrated the samples, and incubated them overnight with the second antibody-AP conjugate. We then detected the second probe by use of INT/BCIP (Boehringer-Mannheim) as a substrate for red color staining. Then, we stopped the reaction with three changes of a buffer containing 1 mM EDTA, fixed the samples with 4% PFA/0.2% glutaraldehyde, and stored them at 4°C pending observation.

Antibodies and Immunostaining

For immunostaining for neurofilaments, culture supernatants of densely grown hybridoma cells producing mAb 2H3 (Mark *et al.*, 1993) were used at a 1/2 dilution. Peroxidase-conjugated affinity-purified goat anti-mouse IgG (Jackson Immuno Research) was used at a 1/100 dilution for detection of 2H3. Whole-mount immunostaining was carried out as described previously (Shimamura *et al.*, 1994). For double-staining by RNA *in situ* hybridization and immunostaining, the latter was carried out after the completion of the *in situ* hybridization steps.

Analysis of Samples

The whole-mount stained samples were observed and photographed with a Nikon SMZ-10 binocular microscope. For sectioning of whole-mount stained samples, they were immersed in a graded series of sucrose solutions and embedded in Tissue-Tek OCT Compound (Miles Scientific). After freezing them in liquid nitrogen, we cut them into 12- μ m cryosections. After the sections had been treated with Tris-buffered saline to remove the OCT compound, they were dehydrated through a graded series of ethanol and xylene. These dehydrated samples were mounted with Entellan (Merck) and observed under a Zeiss Axiophot microscope.

RESULTS

Isolation of Mouse Cad6 cDNA

We isolated overlapping cDNA clones with a 2373-bp ORF (DDBJ Accession No. D82029). The ORF encoded a

protein with 790 amino acids (Fig. 1); it showed 96% identity to each of human cadherin-6 (Xiang *et al.*, 1994) and rat K-cadherin (Shimoyama *et al.*, 1995) and also exhibited 86% identity to chicken cadherin-6B (Nakagawa and Takeichi, 1995). None of the other cadherins thus far identified were more similar to the cloned molecule. We therefore concluded that the isolated molecule is the mouse homologue of cad6.

Cad6 Expression Subdivides the Early Neural Tube

We studied the expression of cad6 mRNA during embryogenesis by *in situ* hybridization. Its staining signal was first observed at the head process stage in the crescent area located at the anterior end of the embryo (Fig. 2A), that forms the anterior intestinal portal. The cad6 expression in this particular structure of the endoderm was maintained at later stages. Then, cad6 appeared in the neural plate: Its expression was initiated at a portion of the hindbrain at around E7.75 (Fig. 2B, arrows) and subsequently extended posteriorly to the caudal end of the forming spinal cord by E8.0 (Fig. 2D). The rostral end of this cad6 expression exhibited a sharp limit in the hindbrain region (Fig. 2D, arrow). In those positive regions of the neural plate, cad6 expression seemed to occur in all neuroepithelial cells, as revealed by sectioning of the samples (Fig. 2F). Soon after the hindbrain expression, the entire forebrain also began cad6 expression, which bordered the midbrain (Fig. 2C, arrow). At the same time, the midbrain and the anterior hindbrain also expressed cad6, but their cad6 expression was confined to the ridge of the neural fold (Fig. 2D).

At the subsequent stages, the above pattern changed dramatically in the posterior hindbrain and spinal cord. In these regions, cad6 expression was downregulated in the neural plate and closing neural tube, leaving only the neural crest zones positive. Exceptionally, however, the cad6 expression persisted in a band-like unit of the hindbrain (Figs. 2C and 2I). To identify this portion as well as the initial cad6 positive area in the hindbrain, we used *Krox-20* transcript as a marker, which is expressed only in rhombomeres 3 (r3) and 5 (r5) (Wilkinson *et al.*, 1989). By double *in situ* hybridization for *Krox-20* and cad6 messages, we found that the anterior limit of the initial cad6 expression delineated the future r4/5 boundary, as it coincided with the anterior limit of the *Krox-20* signals in r5 (Fig. 3A). When cad6 expression was confined to the band, its anterior limit bordered the r5 *Krox-20* signals (Fig. 3B), suggesting that the cad6-positive band corresponds to r6. This r6-specific expression of cad6 persisted until the time of neural tube closure and thereafter diminished (Figs. 4A and 4B). To summarize, cad6 expression in the neural plate occurred initially from the future r4/5 boundary to the caudal end throughout and then was confined to r6.

Cad6-Positive Neural Crest Cells Populate Peripheral Nerves

The expression of cad6 along the ridge of the neural fold suggested that cad6 is expressed in neural crest cells or their

PRE	
m-cad6	MRTYRYFLLLFWGQPYPTFSNPLSKRTSGFFAKKATLELSADSRNELSRSKR
h-cad6	MRTYRYFLLLFWGQPYPTLSPLSKRTSGFFAKKATLELSADSRNELSRSKR
K-cadherin	MRTYRYFLLLFWGQPYPTFSNPLSKRTSGFFAKKATLELSADSRNELSRSKR
c-cad6B	MRTYRYFLLLFWGQPYPTFSNPLSKRTSGFFAKKATLELSADSRNELSRSKR
EC1	
m-cad6	QWNNQFFLLLEETGSDYQYVGLHSDQDRGDSGLKYLLSGDAGDLFIINENIGDIQATKRLDREKPYVILRAQAVNRRTGRVPESEPTIKIHDINNEP
h-cad6	SWNNQFFLLLEETGSDYQYVGLHSDQDRGDSGLKYLLSGDAGDLFIINENIGDIQATKRLDREKPYVILRAQAVNRRTGRVPESEPTIKIHDINNEP
K-cadherin	SWNNQFFLLLEETGSDYQYVGLHSDQDRGDSGLKYLLSGDAGDLFIINENIGDIQATKRLDREKPYVILRAQAVNRRTGRVPESEPTIKIHDINNEP
c-cad6B	SWNNQFFLLLEETGSDYQYVGLHSDQDRGDSGLKYLLSGDAGDLFIINENIGDIQATKRLDREKPYVILRAQAVNRRTGRVPESEPTIKIHDINNEP
EC2	
m-cad6	IPTKDYVYATVPEADVGTFVQVVTATDAADPTGNSAKVYVSYLQGGPYFVSSESTGIITALLNDRENREQYQVVIQAKDGGQGGGLSGTTTNNITLTDVNDNP
h-cad6	IPTKDYVYATVPEADVGTFVQVVTATDAADPTGNSAKVYVSYLQGGPYFVSSESTGIITALLNDRENREQYQVVIQAKDGGQGGGLSGTTTNNITLTDVNDNP
K-cadherin	IPTKDYVYATVPEADVGTFVQVVTATDAADPTGNSAKVYVSYLQGGPYFVSSESTGIITALLNDRENREQYQVVIQAKDGGQGGGLSGTTTNNITLTDVNDNP
c-cad6B	IPTKDYVYATVPEADVGTFVQVVTATDAADPTGNSAKVYVSYLQGGPYFVSSESTGIITALLNDRENREQYQVVIQAKDGGQGGGLSGTTTNNITLTDVNDNP
EC3	
m-cad6	RFPQSTYQFKTPSSPPGTFIGRIKASDAVGNASIEYSITDGEGLMDFVITDQRTQEGILITVKLLDFEKKVYTLKVKASNPVEPRFLYLGPFDASATVRIIVDDVDEPP
h-cad6	RFPQSTYQFKTPSSPPGTFIGRIKASDAVGNASIEYSITDGEGLMDFVITDQRTQEGILITVKLLDFEKKVYTLKVKASNPVEPRFLYLGPFDASATVRIIVDDVDEPP
K-cadherin	RFPQSTYQFKTPSSPPGTFIGRIKASDAVGNASIEYSITDGEGLMDFVITDQRTQEGILITVKLLDFEKKVYTLKVKASNPVEPRFLYLGPFDASATVRIIVDDVDEPP
c-cad6B	RFPQSTYQFKTPSSPPGTFIGRIKASDAVGNASIEYSITDGEGLMDFVITDQRTQEGILITVKLLDFEKKVYTLKVKASNPVEPRFLYLGPFDASATVRIIVDDVDEPP
EC4	
m-cad6	VFSKLAYILQIREDAQINTTISVAADQPDAAARNPVKYSVDRHTMDRIPIINISGNGSIPTSKLLDRETLWNNITVIAEINNPQSSRVPLIYIKVLDVNDNP
h-cad6	VFSKLAYILQIREDAQINTTISVAADQPDAAARNPVKYSVDRHTMDRIPIINISGNGSIPTSKLLDRETLWNNITVIAEINNPQSSRVPLIYIKVLDVNDNP
K-cadherin	VFSKLAYILQIREDAQINTTISVAADQPDAAARNPVKYSVDRHTMDRIPIINISGNGSIPTSKLLDRETLWNNITVIAEINNPQSSRVPLIYIKVLDVNDNP
c-cad6B	VFSKLAYILQIREDAQINTTISVAADQPDAAARNPVKYSVDRHTMDRIPIINISGNGSIPTSKLLDRETLWNNITVIAEINNPQSSRVPLIYIKVLDVNDNP
EC5	
m-cad6	EFAPFYETPVCEKAKADQLIQTLRAVKKDDPYSGHQFSPSLAPEAASSNPTIQDNKNTAGILTRKNGYNRHEMSTYLLPVVISDNDYPQSSSTQTVTVRVACDHRGNIESCHAE
h-cad6	EFAPFYETPVCEKAKADQLIQTLRAVKKDDPYSGHQFSPSLAPEAASSNPTIQDNKNTAGILTRKNGYNRHEMSTYLLPVVISDNDYPQSSSTQTVTVRVACDHRGNIESCHAE
K-cadherin	EFAPFYETPVCEKAKADQLIQTLRAVKKDDPYSGHQFSPSLAPEAASSNPTIQDNKNTAGILTRKNGYNRHEMSTYLLPVVISDNDYPQSSSTQTVTVRVACDHRGNIESCHAE
c-cad6B	EFAPFYETPVCEKAKADQLIQTLRAVKKDDPYSGHQFSPSLAPEAASSNPTIQDNKNTAGILTRKNGYNRHEMSTYLLPVVISDNDYPQSSSTQTVTVRVACDHRGNIESCHAE
TM	
m-cad6	ALIHPTGLSGALVAILLCIVILLVTVVLFAAL
h-cad6	ALIHPTGLSGALVAILLCIVILLVTVVLFAAL
K-cadherin	ALIHPTGLSGALVAILLCIVILLVTVVLFAAL
c-cad6B	ALIHPTGLSGALVAILLCIVILLVTVVLFAAL
CP	
m-cad6	RRQRKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDITGLRNPEAIDSKSRDIPVPEALPLPRRTPTARNTDVRDFINQRLKENDTPTAPPYDSLATYAYEGTGSVADSLSSL
h-cad6	RRQRKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDITGLRNPEAIDSKSRDIPVPEALPLPRRTPTARNTDVRDFINQRLKENDTPTAPPYDSLATYAYEGTGSVADSLSSL
K-cadherin	RRQRKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDITGLRNPEAIDSKSRDIPVPEALPLPRRTPTARNTDVRDFINQRLKENDTPTAPPYDSLATYAYEGTGSVADSLSSL
c-cad6B	RRQRKEPLIISKEDIRDNIVSYNDEGGGEEDTQAFDITGLRNPEAIDSKSRDIPVPEALPLPRRTPTARNTDVRDFINQRLKENDTPTAPPYDSLATYAYEGTGSVADSLSSL
m-cad6	ESVTTDGDQDYLYLSDWGPFRFKLADMYGQMSDKDS * 790 a.a. identity
h-cad6	ESVTTDGDQDYLYLSDWGPFRFKLADMYGQMSDKDS * 790 a.a. 96%
K-cadherin	ESVTTDGDQDYLYLSDWGPFRFKLADMYGQMSDKDS * 789 a.a. 96%
c-cad6B	ESVTTDGDQDYLYLSDWGPFRFKLADMYGQMSDKDS * 790 a.a. 86%

FIG. 1. The alignment of cadherin-6 amino acid sequences from different species. The netted amino acids are conserved among the species. K-cadherin is the rat homologue of cadherin-6. m, mouse; h, human; c, chicken; a.a., amino acids. PRE, precursor; EC, extracellular domain; TM, transmembrane domain; CP, cytoplasmic domain.

precursors, although r3 and r5 were virtually negative for cad6 expression (Fig. 3). As expected from these observations, cells with intense cad6 expression migrated out of the neural plate or tube. The first migration occurred in the midbrain (Fig. 2C), and then in the other regions; cells in r6 migrated out last. Neural crest cells expressing cad6 and derived from r1/2 became arranged into the form of the trigeminal ganglion, and some of them migrated out into the mandibular arch (Figs. 4A and 4B). Crest cells from other regions exhibited streams with a pattern characteristic of each origin (Fig. 4B).

Sections of the whole-mount stained samples revealed the following profiles of the generation and migration of cad6-positive neural crest cells: In the midbrain and anterior

hindbrain, cad6 expression was observed only at the ridge of the neural fold (Fig. 2E); the positive cells then migrated out (Fig. 2H). In the other regions, however, all neural plate cells initially expressed cad6 (Figs. 2F and 2I). Then positive cells progressively became restricted to the ridge of the neural fold. Finally, the cad6-positive crest cells left the fold (Figs. 2J and 4C). Forming cranial ganglia were positive for cad6 expression, especially at their outer peripheral zones (Fig. 4C), but these expressions were transient, as described below.

In sections of E12.0 embryos, the most intense cad6 signals were detected along peripheral nerves (Fig. 5A). The signals were also located beneath the epidermis, exhibiting nerve-like networks (Fig. 5C). When these samples were

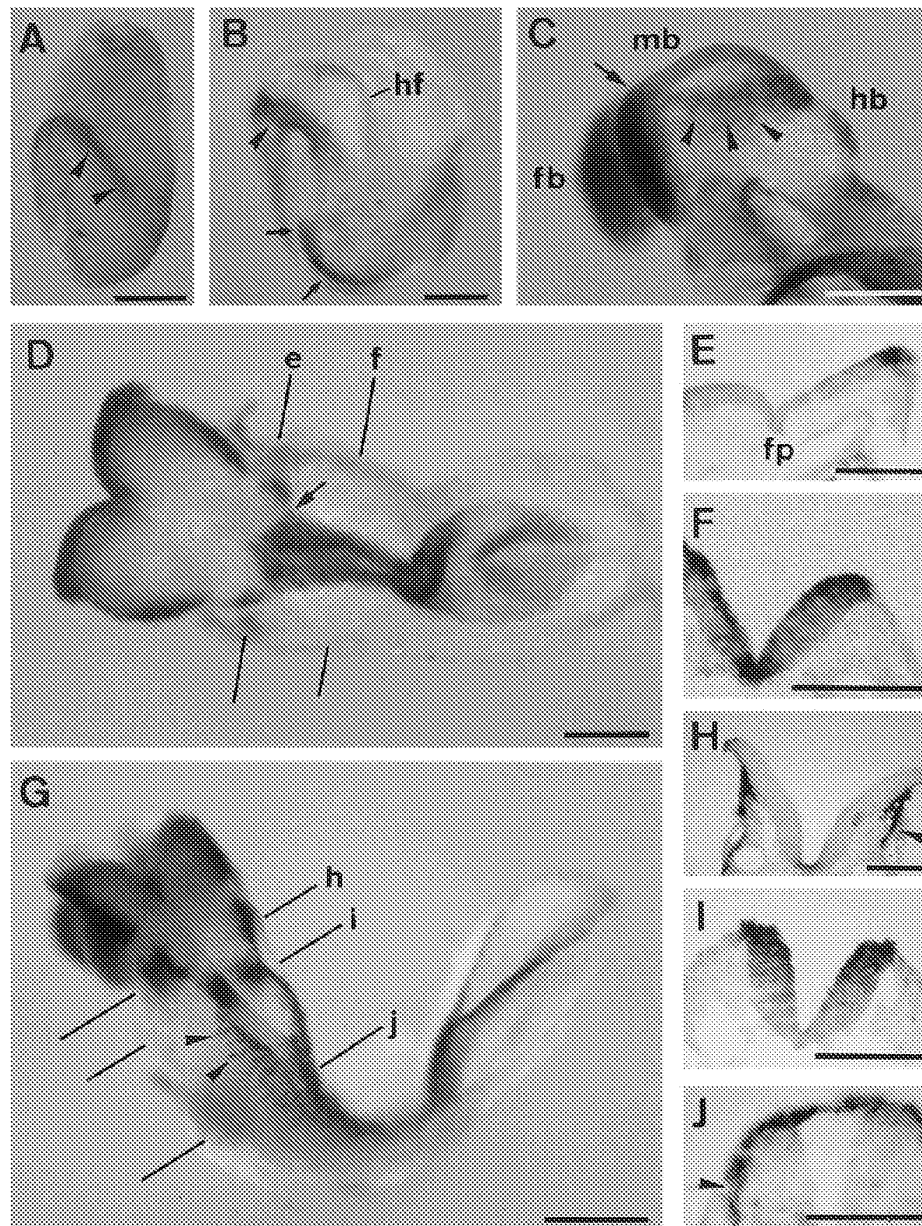


FIG. 2. Cad6 mRNA expression in early embryos. Embryos were stained by *in situ* hybridization as whole-mount samples (A–D, G), and some of them were subsequently sectioned (E, F, H–J). (A) E7.5. The anterior crescent area (arrowheads) expresses cad6. (B) Anterolateral view of an E7.75 embryo. The neural plate has begun cad6 expression at regions indicated by the arrows. The arrowhead indicates the anterior intestinal portal. (C, D) Lateral (C) and dorsal (D) views of an E8.0 embryo. In the neural plate, two borders of cad6-positive areas are observed, at the forebrain/midbrain boundary and in the hindbrain (arrows). Arrowheads point to migrating neural crest cells in the midbrain. (E, F) Frontal sections of the embryo in (D), at the levels of “e” (E) and “f” (F). (G) Dorsolateral view of an E8.5 embryo. A rhombomere-like band in the hindbrain at the level “i,” and streams of neural crest cells (arrowheads) are positive for cad6 expression. (H, I, J) Frontal sections of the embryo in panel G, at the levels of “h” (H), “i” (I), and “j” (J). Arrowheads denote cad6-positive migrating neural crest cells. hf, head fold; fb, forebrain; fp, floor plate; mb, midbrain; hb, hindbrain. Bars, 200 μ m for (A–D, G), 100 μ m for (E, F, H, I), and 10 μ m for (J).

double-stained for cad6 mRNA and neurofilament proteins, most cad6-positive cells were found to be associated with the latter signals (Figs. 5B and 5C), suggesting them to be a group of Schwann cells. For further confirmation of this

identification, we examined cad6 expression in *Sp1otch* mutant mice. In this mutant, Schwann cells migrate into the forelimbs but not into the hindlimbs (Franz *et al.*, 1990). We found that cad6-positive cells were present in the fore-

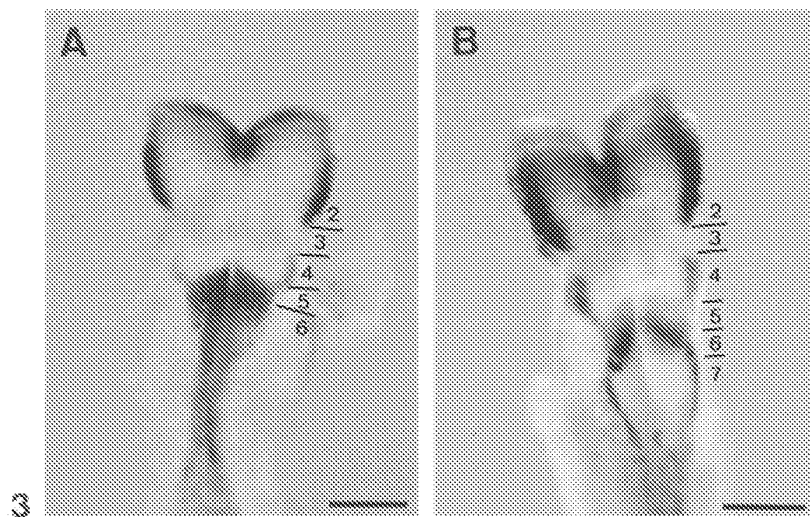
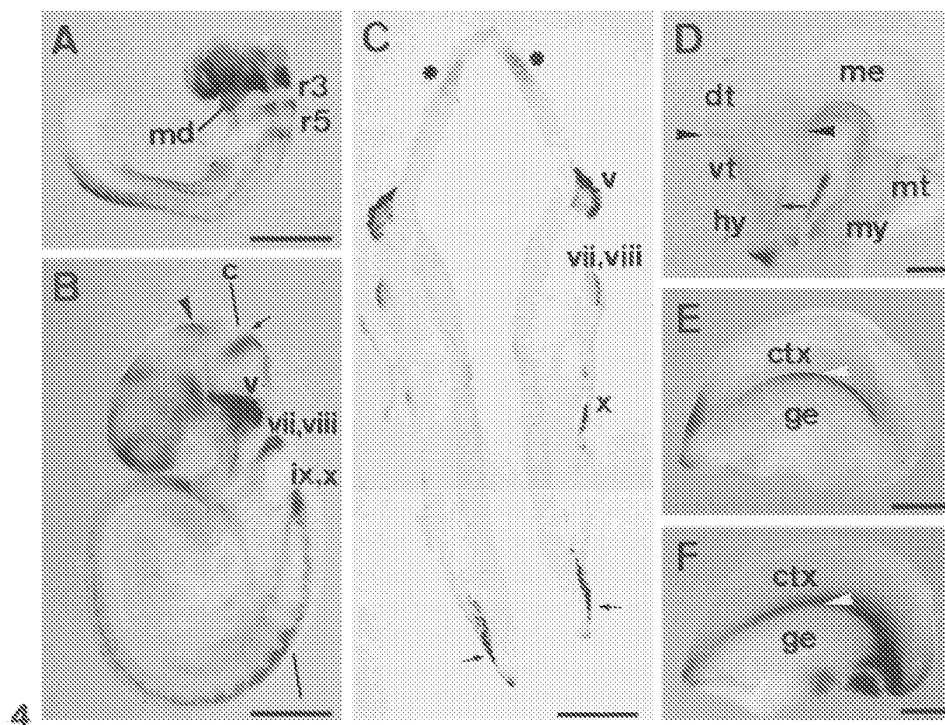


FIG. 3. *Cad6* mRNA expression sequentially defines prospective rhombomere boundaries. Double *in situ* hybridization for *cad6* (blue) and *Krox-20* (red) messages. (A) E8.0. The anterior limit of *Krox-20* signals in rhombomere 5 (r5) coincides with that of *cad6* signals. (B) E8.5. *Krox-20* signals in r5 posteriorly border the *cad6* band. Rhombomere numbers are shown. Short lines indicate prospective rhombomere boundaries. Bars, 200 μ m.

FIG. 4. *Cad6* mRNA expression in migrating neural crest cells and in brains. (A) E9.0. *Cad6*-positive neural crest cells migrate into the mandibular arch. (B) E9.5. *Cad6* signals are observed in forming cranial ganglia and in nerves, as well as in migrating crest cells in the trunk. In the brain, the signals are seen at the anterior border of the dorsal hindbrain (arrow) and in a dorsal portion of the midbrain (arrowhead). (C) A longitudinal section of the embryo in (B), which was cut along level "c" depicted in (B). Asterisk, anterior hindbrain signals; arrows, migrating *cad6*-positive crest cells in the trunk. (D) A dissected brain of E12.5 embryo. The brain was longitudinally cut into half and observed from the ventricular side. The cerebral hemisphere was removed in this sample. The arrow indicates the alar/basal boundary, and arrowheads point out the dorsal/ventral thalamus boundary. (E, F) Ventricular view of an isolated E12.5 telencephalon, hybridized for *cad6* (E) and R-cadherin (F). White arrowheads mark the border of the cortex to the lateral ganglionic eminence. md, mandibular arch; v, trigeminal ganglion; vii, viii, facial/acoustic ganglion; ix, x, glossopharyngeal/vagus nerves; dt, dorsal thalamus; vt, ventral thalamus; hy, hypothalamus; me, mesencephalon; mt, metencephalon; my, myelencephalon; ctx, cerebral cortex; ge, ganglionic eminence. Bars, 0.5 mm for (A, B, D-F) and 200 μ m for (C).

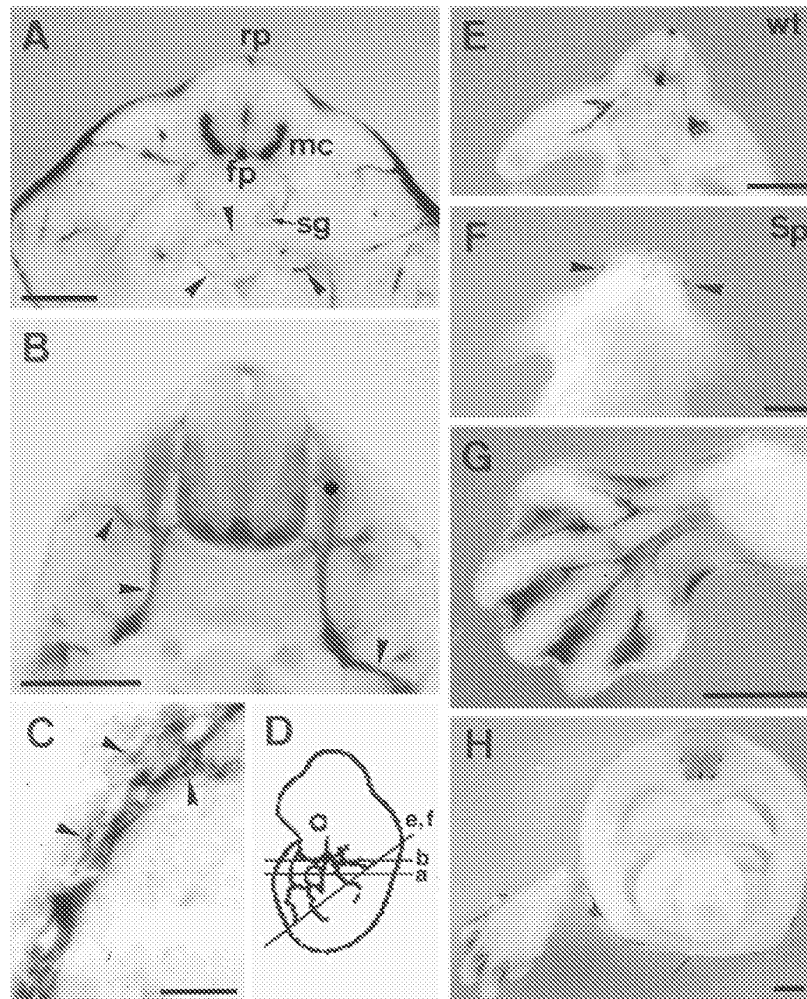


FIG. 5. Cad6 mRNA expression in peripheral nerves and other tissues. (A) Slice of an E12.0 embryo at the level of "a" in (D). Positive regions include nerve tracts, a peripheral zone of the sympathetic ganglia, roof plate, floor plate, and a portion of the motor column (mc). Neural crest-derived cells around the trachea, artery, and esophagus (arrowheads) also express cad6 mRNA. (B) Double-staining for cad6 mRNA (blue) and neurofilament proteins (brown) on a slice of an E12.0 embryo at the level of "b" in (D). Dorsal root and its ganglia (asterisk) are negative for cad6 expression, whereas other peripheral nerve fibers show both signals (arrowheads). (C) High magnification of a peripheral nerve stained as in (B). Note that cad6-positive cells (arrowheads, blue) are associated with neurofilament-positive fibers (brown). (D) Drawing of an E12.0 embryo, showing the levels of sections. (E, F) Cad6 expression in E12.0 *Splotch*^{+/+} (E) and *Splotch*^{-/-} (F) embryos at the level of "e/f" in (D). Cad6-positive nerves are absent in the hindlimbs of the homozygous embryo. Arrowheads indicate cad6 signals at the edge of the open neural tube in the mutant. (G) A hindlimb of an E12.0 embryo. Interdigital portions and nerve fibers running underneath the epidermis are positive. (H) A slice of E15.0 embryo showing absence of cad6 signals in the peripheral nerves. fp, floor plate; mc, motor column; rp, roof plate; sg, sympathetic ganglion. Bars, 400 μ m for (A, B, E-H) and 20 μ m for (C).

limbs but not in the hindlimbs (Figs. 5E and 5F), consistent with the above identification. In the caudal spinal cord of the *Splotch* mice, which fails to close, cad6 signals were detected at the edges of the open neural plate. This suggests that cad6-positive neural crest precursors formed but did not migrate out from these positions in the neural plate.

Not all of the peripheral nerves were positive for cad6 expression; e.g., the dorsal root was negative. Also, cranial ganglia, dorsal root ganglia, and sympathetic ganglia were negative at least in their central portions when their differentiation

had been completed, although some peripheral signals were observed. Most of these nerve-associated expressions of cad6 were no longer evident in E15.0 embryos (Fig. 5H). In addition, we found that, in the limbs, interdigital mesenchyme expressed cad6 in a characteristic pattern (Fig. 5G).

Cad6 Expression Delineates Fetal Brain Subdivisions

The expression pattern of cad6 in the brain dynamically changed during development. By E9.5, the cad6 expression

in the forebrain had become regional (Fig. 4B); positive regions included the dorsal midline, the future neural retina and optic stalk, and portions of the diencephalon. It also occurred in a dorsal area of the midbrain and dorsally at the rostral limit of the hindbrain (Fig. 4B). In the brain of E12.5 embryos, cad6 expression was found in the dorsal portion of the ganglionic eminence, and this signal sharply bordered the lateral cortex (Fig. 4E). The latter expresses R-cadherin (Fig. 4F), as was noted earlier (Matsunami and Takeichi, 1995); therefore, these two cadherins showed a complementary pattern at the boundary of the two contiguous cerebral compartments. Besides, a faint continuous line of cad6 signals occurred at the zona limitans intrathalamica along the boundary between the dorsal and ventral thalamus and along the alar/basal boundary in the diencephalon and midbrain (Fig. 4D). Hypothalamic compartments were also positive. All these staining signals emanated from the ventricular neuroepithelial cells. In the spinal cord, a certain portion of motor columns as well as the roof and floor plates expressed this cadherin (Figs. 5A, 5B, and 5E); the floor plate signals extended to the brain. In the eye, the future iris and optic stalk were positive (data not shown). At later developmental stages, cad6 expression in the brain underwent further complex changes following differentiation of brain nuclei, and these will be reported elsewhere (S. C. Suzuki, T. Inoue, and M. Takeichi, in preparation).

Cad6 Expression in *Hoxa-1*^{-/-} Mice

Since the cad6 expression pattern in the early hindbrain was in part reminiscent of the well-known patterns of Hox gene expressions, we considered the possibility that the former might be regulated by the latter genes. As an initial step for testing this possibility, we examined the effect of *Hoxa-1* knockout on the expression of cad6.

We first carried out whole-mount *in situ* hybridization for *Hoxa-1* expression in wild-type E8.0 embryos to confirm the previous observation that this gene is expressed posteriorly to the presumptive r3/4 boundary in the hindbrain (Murphy and Hill, 1991). As reported, the *Hoxa-1* expression exhibited a sharp anterior limit within the hindbrain. In four-somite stage embryos, however, the limit was located more posteriorly to the r3/4 boundary, although it covered a posterior portion of r4 (Fig. 6A, 4s). In nine-somite stage embryos, the expression was diminishing, but it was still positive to a certain degree (Fig. 6A, 9s).

We then examined cad6 expression in E8.0 (2- to 4-somite stage) *Hoxa-1*^{-/-} embryos (Chisaka et al., 1992) and found that they expressed this molecule in a pattern similar to that in wild-type embryos. However, close comparisons of the mutant and wild-type samples revealed that the staining intensity for cad6 messages was considerably reduced in the posterior hindbrain of the mutants (Fig. 6B). Moreover, the anterior limit of the expression was less sharp and located more posteriorly in the mutants than in the wild-type, as estimated by distances from the position of the anterior-most somite and from a sulcus corresponding to the presumptive r2/3 boundary. The latter distance was larger in

the mutants, despite their shorter hindbrain due to the absence of r5 (Carpenter et al., 1993). At the 9-somite stage when the discrete r6 band of cad6 appears in the wild-type embryos, the corresponding band was dim in the mutants (Figs. 6C-6F). Furthermore, cad6 expression at the neural fold ridge of the putative r4 region was faint (Figs. 6C-6F). The other cad6 expressions were, however, basically normal. At the 11-somite stage, cad6 appeared at the neural fold ridge of the putative r4 and r6 regions (Figs. 6G and 6H), although the main portion of the neural plate remained essentially negative. These cad6 signals were contiguous to each other on the ridge, consistent with the previous finding that r5 is missing in *Hoxa-1*^{-/-} embryos (Carpenter et al., 1993; Mark et al., 1993; Dollé et al., 1993). Curiously, cad6 signals in r2 extended more caudally in the mutant embryos than in normal embryos (Figs. 6F and 6H), although no such effect of *Hoxa-1* mutations has been reported. In E9.5 homozygous mutant embryos, neural crest cells expressing cad6 migrated out of the putative r4 and r6 positions (Fig. 6J). The two clusters of cad6-positive migrating crest cells fused to each other over the dorsal portion of the otic vesicle, as reported previously (Mark et al., 1993). Thus, the suppression of cad6 expression in the posterior hindbrain by *Hoxa-1* mutation was limited to earlier developmental stages. Importantly, the observed effect of *Hoxa-1* mutation coincides with the period of the expression of this gene. We performed similar analysis for *Hoxa-3* mutants, but found no effect on cad6 expression in them (data not shown).

DISCUSSION

Recently, we reported the cloning and expression of chicken cadherin-6B (cad6B; Nakagawa and Takeichi, 1995). The mouse cad6, studied here, showed 86% identity in amino acid sequence to the cad6B, so they can be considered to be homologs of each other. Despite this high similarity of the two molecules, their expression patterns were not identical, raising a question about their relationship. The chicken cad6B was expressed only in the neural fold, whereas the mouse cad6 was expressed in both the neural fold and migrating neural crest cells; this was the reason why the chicken molecule was termed cad6B instead of cad6. We found another chicken cadherin, cadherin-7 (cad7); this cadherin is expressed in migrating crest cells but not in the early neural plate and tube (Nakagawa and Takeichi, 1995). Therefore, in a sense, the expression pattern of cad6 represents a mixture of those of cad6B and cad7. However, the cad6 expression pattern was not a simple sum of the two chicken cadherin patterns; it was different in certain details. For example, neither of these chicken cadherins displayed a metamer expression in the hindbrain neural plate. In the chicken, other unidentified cadherins might be present to subdivide the hindbrain.

Possible Roles for cad6 in Neural Tube Segmentation

It was shown earlier that neuroepithelial cells in chicken embryos cannot cross the boundaries between rhombomeres

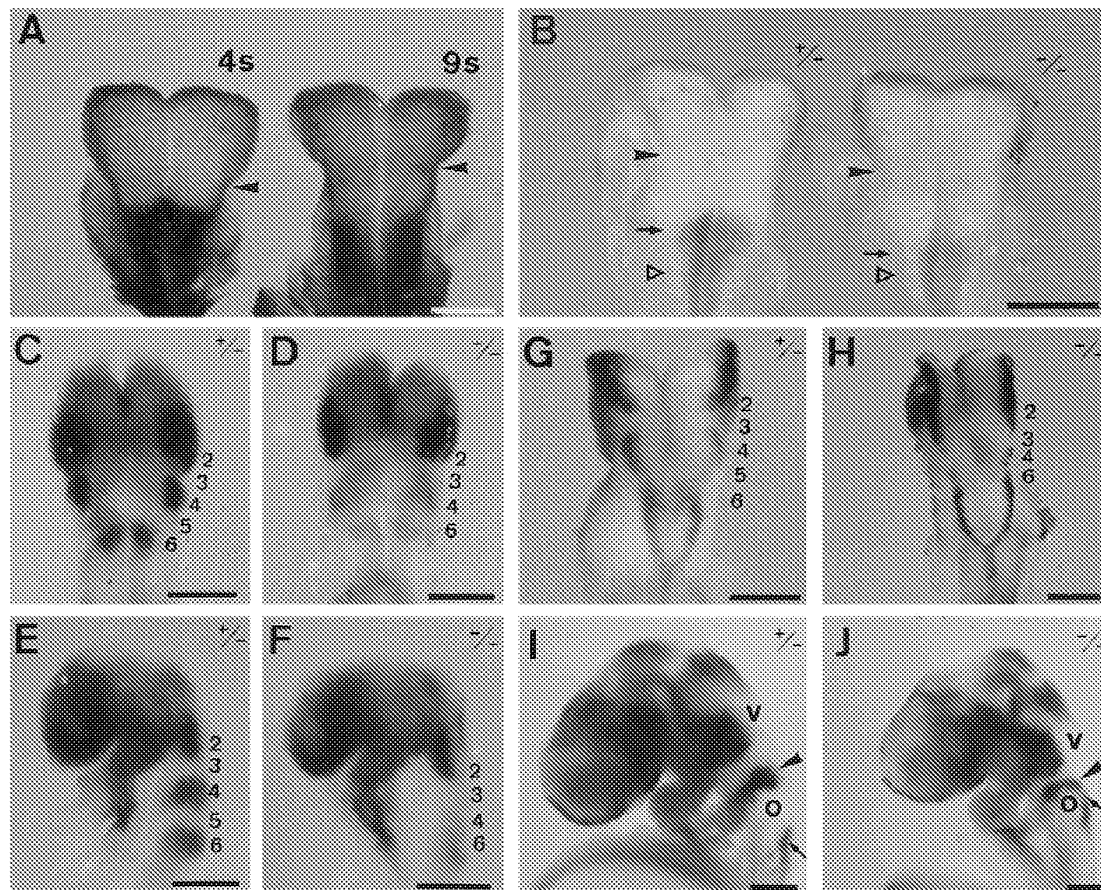


FIG. 6. Effects of *Hoxa-1* mutation on *cad6* mRNA expression. Dorsal views for A–D, G, and H and lateral views for E, F, I, and J. (A) *Hoxa-1* expression in 4-somite (4s) and 9-somite (9s) embryos. Intense signals in the 9s hindbrain are mostly derived from mesenchymal tissues. (B) *Cad6* expression in *Hoxa-1*^{+/+} (left) and *Hoxa-1*^{-/-} (right) embryos at the 4s stage. Triangles indicate the gap between the first and second somite blocks. Arrows show the anterior limit of *cad6* expression. Arrowheads point to the sulcus corresponding to the future r2/3 boundary in both panels of (A) and (B). Note the posterior retreatment of the *cad6*-positive region in the mutant hindbrain. In these samples, *cad6* expression had not fully developed in the r4 and more anterior portions. (C–F) E8.5 (9-somite stage). (C, E) *Hoxa-1*^{+/+} and (D, F) *Hoxa-1*^{-/-}. In the mutant, *cad6* signals in r4 and r6 are greatly reduced; positive signals visible at r4 in B are mostly derived from ventral tissues. (G, H) E8.5 (11-somite stage). (G) *Hoxa-1*^{+/+} and (H) *Hoxa-1*^{-/-}. Some *cad6* signals appeared in the putative r4/r6 region on the ridge of the neural plate; however, the signals in the main portion of the r6 neural plate remain very faint or negative. (I, J) E9.5. (I) *Hoxa-1*^{+/+} and (J) *Hoxa-1*^{-/-}. Two streams of *cad6*-positive cranial ganglion cells, derived from r4 (arrowheads) and r6 (arrow), have fused with each other over the otic vesicle (o), v, trigeminal ganglion. Prospective rhombomere numbers are shown. Bars, 200 μ m.

after a certain developmental stage (Fraser *et al.*, 1990), except that a minor population of them can do (Birgbauer and Fraser, 1994). A hypothesis to explain this phenomenon was that the restriction is created by immiscibility of cells belonging to contiguous rhombomeres (Lumsden, 1990; Wingate and Lumsden, 1996). Our present finding supports this hypothesis. After dynamic changes in expression pattern, the *cad6* signals became restricted to rhombomere 6 (r6), although temporarily. We recently showed that cells expressing *cad6B* segregate from cells expressing other cadherins, such as N-cadherin and *cad7*, upon their aggregation *in vitro* (Nakagawa and Takeichi, 1995). Such adhesion specificity was also found for mammalian *cad6* (S. C. Suzuki and M. Takeichi, unpublished). Based

on these observations, we can assume a process such that r6 cells coaggregate by use of *cad6*, and segregate from cells of contiguous rhombomeres without this cadherin; the latter probably express other unidentified cadherins. This is a likely mechanism for prohibiting r6 cells from free migration to the contiguous rhombomeres. Grafting experiments suggested that cells derived from different odd-number rhombomeres are miscible with one another but not with those from even ones (Cuthrie *et al.*, 1993). To be consistent with this observation, odd or even rhombomeres should express the same cadherins, but this was not the case in the present observation. Other cadherins that can crossinteract with *cad6* might be expressed in even rhombomeres. A combinatory action of

multiple cadherins is perhaps important for determining the overall adhesive behavior of rhombomere cells. For testing these hypotheses, it would be important to identify other cadherins expressed in the early hindbrain. N-cadherin is known to be expressed in the neural tube, but its expression in the hindbrain is not so intense yet at the stage of the unclosed neural plate (Kimura *et al.*, 1995).

We should point out that cad6 expression in the hindbrain dynamically changes. Its anterior limit initially delineated the prospective r4/5 boundary and subsequently the r5/6 boundary. This pattern implies that cad6 could be involved in determining both boundaries in a sequential manner. An important question is whether these cad6 expressions indeed correlate with cell migration restriction at these boundaries. No precise cell lineage analysis has been reported for the early mouse hindbrain except for neural crest origin (Osumi-Yamashita *et al.*, 1996), so that this question remains to be answered.

In brains at more advanced developmental stages, we found other correlations between cad6 expression pattern and cell migration restriction. An example was seen in the developing telencephalon. Cad6 expression delineated the dorsal border of the ganglionic eminence, which is contiguous to the R-cadherin-expressing lateral cortex (Matsunami and Takeichi, 1995). These two cadherins showed a complementary distribution pattern at the junction of the ganglionic eminence and cortex. It was previously shown that cells in the ganglionic eminence do not migrate into the cortex (Fishell *et al.*, 1993), and we also demonstrated that R-cadherin-positive and -negative fetal brain cells segregate from one another in an *in vitro* aggregation system (Matsunami and Takeichi, 1995). Furthermore, a weak cad6 expression occurred along the boundaries between the dorsal and ventral thalamus; it was demonstrated that cells do not cross the corresponding boundary in the chicken embryo (Figdor and Stern, 1993). A similar type of expression patterns delineating boundaries in the embryonic brain were also recently reported for *Xenopus* F-cadherin (Espeseth *et al.*, 1995). These findings support the hypothesis that regional expressions of multiple cadherins in the fetal brain play a role in the maintenance of its segmental structure (Gänzler and Redies, 1995; Matsunami and Takeichi, 1995).

Subpopulation-Specific Expression of cad6 in Neural Crest Cells

We found that cad6 is expressed along the ridge of the neural fold and subsequently in migrating neural crest cells. The cad6-positive cells in the ridge are most likely neural crest precursors. It thus seems that neural crest cells begin cad6 expression when they are still part of the neural plate, and subsequently they migrate out. Most of the cad6-positive crest cells selectively accumulated along nerve fibers, suggesting that they differentiated into certain types of Schwann cells. It remains to be investigated whether this subpopulation-specific cad6 expression is established from the beginning of neural crest generation or during their migration. Whatever the case is, cad6 may contribute to the

clustering of migrating Schwann cell precursors and/or their sorting from other cells, as we proposed recently (Nakagawa and Takeichi, 1995). Since motor neurons also express cad6, this cadherin could also be used for the association of Schwann cells with motor axons.

It is interesting to note that cad6 expression was virtually negative in r3 and r5 of E8.5 embryos. These odd rhombomeres are known to produce neural crest cells, which migrate together with others from even rhombomeres (Sechrist *et al.*, 1993; Birgbauer *et al.*, 1995). It remains to be resolved whether these r3/r5-derived crest cells express cad6 when joining those derived from even rhombomeres. In case they never express cad6, migrating crest cells are assumed to be a mixture of cad6-positive and negative cells; if so, this situation could be effective for segregating subpopulations of migrating crest cells from one another, as discussed above and before (Nakagawa and Takeichi, 1995). In addition, it is worthy of note that cad6 can be used as a marker for neural crest cells, at least for their subpopulations, in the future studies; for the mouse system, such markers have been lacking.

Hoxa-1 Mutation Affects cad6 Expression

As is well known, *Hox* genes are expressed in the neural tube along its anteroposterior axis, and many of them exhibit their anterior limit at specific rhombomere boundaries (Wilkinson, 1993). The early pattern of cad6 expression in the neural plate and tube is reminiscent of those *Hox* expression patterns. This correlation prompted us to test the possibility that some of the *Hox* genes may be regulating cad6 expression. We examined the effect of *Hoxa-1* and *Hoxa-3* mutations on cad6 expression, and found that, in *Hoxa-1* mutants, the cad6 expression pattern was altered in a developmental stage-specific manner. In these mutants at earlier developmental stages, cad6 expression was suppressed at r4 and r6. The cad6 expression in these rhombomeres was, however, upregulated afterward, and crest cells migrating from these regions expressed a normal level of cad6. According to previous (Murphy and Hill, 1991) and present observations, *Hoxa-1* is expressed in E8.0 hindbrains, posteriorly to the r3/r4 boundary. The *Hoxa-1* messages then diminish from the developing brain, and migrating neural crest cells do not express *Hoxa-1*. Thus, both positionally and temporally, the *Hoxa-1* expression pattern in normal embryos correlates with the observed major effects of the *Hoxa-1* mutation on cad6 expression; that is, the effects were limited to rhombomeres posterior to the r3/r4 boundary and up to the stage E8.5. It should be stressed that, in the *Hoxa-1* mutant strain used in the present study, r4 and r6 are present, as revealed by use of specific regional markers, although r4 seems to be reduced in size (Carpenter *et al.*, 1993). It is therefore most likely that the downregulation of cad6 is not due to a simple loss of these rhombomeres. These findings support the idea that *Hoxa-1* might regulate cad6 expression at restricted portions and stages. Other genes perhaps take over *Hoxa-1* to sustain the later cad6 expressions, when the *Hoxa-1* expression is turned off.

However, the present study cannot eliminate the possibility that loss of *Hoxa-1* was not directly linked with the changes in the cad6 expression pattern. Gross morphological changes or some noncharacterized phenotypic transformation of rhombomeres in the mutant hindbrain could have indirectly led to the alterations in cad6 expression. Thus, whether *Hoxa-1* activity directly regulates cad6 expression remains to be further investigated. Obviously, *Hoxa-1* cannot be the sole regulator for cad6 expression, since the expression pattern of the latter is much more complex than that of the former, and also the spinal cord expression of cad6 was not affected by *Hoxa-1* mutation. Multiple positive and negative regulators should be involved in the transcription of the cad6 gene in a position-specific manner. Another Hox gene, *Hoxd-9*, was found to regulate the expression of a cadherin, L-CAM, in an *in vitro* system (Goomer *et al.*, 1994).

In summary, cad6 is expressed in various local regions of the developing mouse brain and in subpopulations of neural crest cells. These regional cad6 expressions likely serve for sorting of heterogeneous cells to establish and maintain the regionalized structure of the body. *Hoxa-1* gene could be one of the regulators for the spatiotemporal expression of cad6 at early developmental stages.

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